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## A SYSTEMATIC STUDY OF THE ACTION OF DEFINITELY RELATED CHEMICAL COMPOUNDS UPON ANIMALS.

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(Continued from p. 307, Vol. XIII.)

### *Nitroprusside of Sodium.*

In the frog this salt produces sluggishness, jerkings, convulsive movements, a diminution of reflex activity, slowing of the respirations and heart-beats, a diminution of sensibility, loss of voluntary motion and, finally, death. The muscles and sensory nerves are depressed, but the motor nerves are not affected. The alterations in reflex excitability are due in part to the action on the sensory nerves, but chiefly to a direct action on the spinal cord. The convulsive movements are spinal in origin.

Given to the dog by the stomach in the dose of 0.2 gram per kilo., there is noted vomiting, salivation, muscular weakness, diminution and loss of tactile sensibility, defecation, unconsciousness, and slight tremors. The respirations are increased, the temperature falls decidedly, the pulse is at first increased, then diminished, clonic or tetanic convulsions may occur, the animal finally passes into a condition of paralysis and general collapse, and within forty-eight hours death ensues from failure of the heart or respiration. The blood is much darkened.

When the effects on the circulation and respiration are studied by means of the kymographion, and the poison is given by the vein in doses of 0.25–0.5 gram, the pulse is at first increased, then decreased, the arterial pressure is lowered, and the respirations are diminished. Repeated doses cause a progressive failure of all three, and death ensues from cardiac or respiratory arrest. The fall of pressure and the alterations in the pulse are due to a direct action on the heart. The cause of the failure of respiration is a direct action on the respiratory centres.

The fatal dose by the stomach is about 0.2 gram per kilo. of body-weight; by the vein, from 0.1–0.2 gram per kilo.

*Nitroso-β-naphthol.*

In the frog, no special symptoms are developed apart from a general and progressive condition of paralysis of all functions. Shortly after the injection, voluntary movements, respiration, and the heart's action become sluggish; voluntary motion is soon lost; the motor reflexes are weakened and finally abolished. The heart is arrested in diastole and is engorged with blood. The motor and sensory nerves and muscles remain normally irritable after death, but the spinal cord is depressed. The diminution and final loss of reflex excitability are due to the action on the spinal cord.

Given to the dog by the stomach, vomiting occurs within a few minutes, and the drug is ejected before sufficient time has elapsed for much to have been absorbed. Other symptoms observed are quietude, sluggishness in voluntary movements, diminution in reflex excitability, a decrease in the pulse-rate, a primary increase followed by a fall of the bodily temperature, and, generally, an increase in the respiration-rate, although there may be a diminution. If the dose has been sufficient to prove fatal, the animal passes into a condition of general failure of all functions, death resulting from respiratory arrest. The blood is discolored, being very dark, and exhibiting by means of the spectroscope the presence of nitrite of hemoglobin.

When injected into the external jugular vein in doses of 0.12–0.25 gram, there usually occurs a trifling increase in the pulse-rate, which is followed by a diminution, or the pulse may at first be unaffected and then decreased, the pressure is lowered, and the respiratory movements are increased. Repeated doses decrease the pulse-rate and pressure and, finally, depress the

respiratory centres. Although the heart is depressed early in the poisoning and the respiratory centres late, death is always due to a paralysis of the latter.

After section of the pneumogastric nerves the increase in the number of respirations does not occur, showing that the action is on the pulmonary peripheries of these nerves. The decrease late in the poisoning is, however, dependent upon a direct depression of the centres.

The alterations in pulse and pressure are observed just the same after section of the vagi and upper cervical portion of the spinal cord, and must, as a consequence, be due to direct actions on the heart.

The fatal dose when injected subcutaneously is about 0.4 gram per kilo., and is relatively very large, owing to the slowness with which absorption occurs. The minimum fatal dose, injected intravenously, is about 0.04 gram per kilo. It is impossible to determine the exact fatal dose when given by the stomach, because of persistent vomiting.

*Nitro-methane.*

No special symptoms are produced in the frog save those attending an increasing condition of depression of all functions: the reflexes are gradually diminished, voluntary motion is lost, the heart's action and respiration become slower and slower, and death ensues. The muscles and sensory nerves are unaffected, but the motor nerves and spinal cord are depressed, especially the latter. The heart is arrested in diastole and is unirritable.

In the dog the hypodermic injection of from one-half to one cc. per kilo. of body-weight will induce death within twenty-four hours. The injection is followed by lassitude, drowsiness, weakness, salivation, urination, defecation and vomiting. The pulse-rate is increased and finally slowed; the temperature is slightly increased during the first hour and then diminished, or it may be decreased from the first; the respirations are variably affected during the early hours, sometimes being unaffected, at other times increased, and at others diminished, but finally are invariably diminished. The motor reflexes gradually weaken, sleep becomes deeper, and, although the animal may be aroused, he immediately relapses; paralysis occurs in the hind extremities, movements cause pain, tremors may be noted, and clonic convulsions occur.

Sleep passes into stupor and then into coma, the respirations fail, and the heart ceases to beat.

Autopsies show that the heart is arrested in diastole, and that the gastro-intestinal mucous membrane is greatly congested and swollen—indicating that death was due to some extent, at least, to this effect.

Special studies made of the actions on the circulation and respiration show that the intravenous injection of about one-half cc. per kilo. always increases the pulse-rate, although this may be preceded by a transient diminution. Even after repeated doses the increase persists and is noted up to the time of death. The arterial pressure is always increased, but this may be preceded by a decline, which is accompanied by a decrease in the pulse, although the latter may be observed without the former. After section of the cardio-inhibitory fibres connecting the corresponding centres with the heart, the same changes in the pulse-rate occur, showing that the action must be either on the heart or accelerator centres. After section both of the vagi and upper cervical portion of the spinal cord, by which the accelerator centres are functionally destroyed, the number of heart-beats is no longer increased as in normal animals. From these facts it is obvious that the increase is due to a stimulation of the accelerator centres. The decrease in the rate which is sometimes observed immediately after the injection and at times just before death is of cardiac origin, because it is noted after the isolation of the heart from the nerve-centres.

The rise of arterial pressure does not occur after section of the upper cervical part of the spinal cord and, therefore, must be due to an action on the vaso-constrictor centres. After very large doses these centres are paralysed.

The respirations are always increased after intravenous injections, and equally well after section of the vagi, proving that this effect is due to a stimulation of the respiratory centres. After several doses this condition gives way to depression, and death occurs from their paralysis long before the circulation shows any appreciable signs of impairment.

The minimum lethal dose is from one-half to one cc. per kilo. of body-weight.

### *Nitroso-diethylene.*

Immediately after the injection of this substance into the posterior lymph-sac of the frog the animal's movements become weakened, voluntary motion is lost, the motor reflexes are rapidly diminished and lost, respiratory movements cease, and the heart's action becomes slower and slower and, finally, is arrested. The muscles and sensory nerves retain their normal irritability, but the spinal cord and motor nerves are depressed. The diminution and loss of reflex irritability are due chiefly to the depression of the spinal cord. The heart is arrested in diastole and is unirritable.

In the dog, after the subcutaneous injection of from one-half to one cc. per kilo. of body-weight, there occur salivation, vomiting, lassitude, urination, defecation, weakness, staggering, diminution of reflex activity, occasional howls and whining as though there were paroxysms of pain, dilatation of the pupils, loss of volitional movement, and unconsciousness. The bodily temperature is decreased; the pulse-rate is also lowered, and the respirations gradually fail, although there may be a more or less marked increase at first. Late in the poisoning the vomited matter may be of a distinctly reddish color.

Death occurs from a failure of respiration; the heart is arrested in diastole; and the mucous membrane of the stomach and intestines is swollen and congested.

After the intravenous injection of somewhat smaller doses, and by special studies made of the effects on the pulse and respiration, we have found that the pulse-rate is diminished, the arterial pressure lowered, and the respirations usually at once increased. Should sufficient of the drug be given to be fatal, all three fail, and death occurs from the arrest of respiration.

After section of the pneumogastric nerves we have noted the curious result that, instead of there being a decrease in the pulse-rate, there was always a marked increase—thus indicating that the drug acts simultaneously in opposite ways to affect the rate. The explanation of the causes of these different effects seems apparent in the results which occur in animals in which the accelerator centres are destroyed. Under such circumstances the increase is no longer observed, showing therefore that this effect is due to a stimulation of the accelerator centres. The decrease noted in intact animals appears to be due to a stimulation of the cardio-

inhibitory centres, and the reason why an increase is not manifest is because the effects of the stimulation of these centres are more than sufficient to counterbalance those of the accelerator-centres. Moreover, the action on the former is assisted by another directly on the heart, for it has been found that after the isolation of the heart from the nerve-centres the number of beats is invariably lessened, especially so late in the poisoning. The decrease of pressure is partly due to a depression of the heart and partly to a similar action on the vaso-motor centres and peripheries. The changes in the respiration-rate are due to direct actions on the corresponding centres—first stimulating and then paralysing them.

The minimal fatal dose injected subcutaneously is less than one cc. per kilo. of body-weight; and intravenously, from one-third to one-half cc. per kilo.

*Hydroxylamine.*

The injection of hydroxylamine in the case of the frog causes excitement, the animal jumping about more or less continually in a purposeless way; reflex excitability is increased, the slightest touch inducing violent movements; muscular twitchings are present. After awhile all movements are impaired; voluntary motion is lost; there is unconsciousness; the muscles are relaxed, the animal lying sprawled out, although the reflexes are still intact; respiration ceases; the heart's action grows feeble and, finally, stops. The nerves and muscles retain their normal irritability even after death, and the spinal cord seems to be the seat of the reflex disturbances. The heart is arrested in diastole, and the blood is everywhere very dark, almost black.

In the dog, after doses by the stomach of 0.1–0.2 gram per kilo. of body-weight, there occur vomiting, salivation, defecation and urination, weakness, muscular twitchings, convulsive movements of a tonic or clonic character, unconsciousness, an increase in the heart-beats, a diminution of temperature, and sometimes an increase, and at others a decrease, in the respiration-rate. The mouth and gums are distinctly cyanotic. Should the dose be larger and fatal the animal passes into a condition of general paralysis, and death results from a failure of respiration. The blood is everywhere very dark.

Careful studies of the actions on the circulation and respiration by the aid of the kymographion show that when the drug is

injected intravenously in doses of about 0.25 gram the arterial pressure is gradually diminished, although there may occur a slight primary increase; the pulse is usually increased in frequency, and feebler, but the number of beats may be subnormal throughout; the respirations are diminished. We have determined that the alterations in both the pulse and pressure are due to a direct action on the heart, and that the failure of the respirations is the result of a direct depression of the respiratory centres.

The discoloration of the blood is due to the formation of methemoglobin.

The lethal dose by the stomach for the dog is about 0.2–0.3 gram per kilo. of body-weight; and by the vein, 0.06 gram per kilo.

*Aldehyde-ammonia.*

In the frog this substance causes hyper-excitability, restlessness, loss of voluntary motion, diminution of reflex excitability, paralysis, and death. The increase of reflex excitability is due to a direct action on the spinal cord, and the diminution to spinal depression, combined with a depression of both sensory and motor nerves. The muscles do not seem to be affected.

When given to the dog by the stomach, vomiting occurs so readily that the poison is ejected before time has elapsed for any appreciable absorption; but if injected subcutaneously, in the dose of 0.15–0.20 gram per kilo., there is considerable pain induced at the seat of injection, the muscles in the region are decidedly stiffened, and the animal's movements are as though the parts were in splints. This is particularly noticeable if the injection is made in the fleshy portion of one of the legs. The dog is restless, and whines as though in pain; salivation may be present; respiration is increased; the temperature is increased from 0.2°–0.4° C., and then falls below the normal; the heart-beats are at first increased, but after a time are always less than the normal. Weakness and unconsciousness ensue, attended with a diminution of reflex excitability, and death occurs within 24 hours, the respiration ceasing before the pulse.

When injected into the circulation in doses of 0.25 gram, the arterial pressure, pulse and respiration are increased. The increase of the pulse is usually of a transient character and is soon followed by a diminution; and the respirations are doubled in frequency. After repeated doses—or a single large dose—pressure, pulse and

respiration rapidly fail, and death results primarily from the paralysis of the respiratory centres. By appropriate methods it has been determined that the changes both in pulse and pressure are dependent upon a direct action on the heart, and that the effects on the respirations are the result of a direct action on the corresponding centres. Aldehyde-ammonia is therefore a primary stimulant and secondary depressant to the circulation and respiration; the actions correspond in essential respects with those of the ammonia salts in general.

The lethal dose, subcutaneously injected, is from 0.15–0.20 gram per kilo. of body-weight; and by the vein, about 0.04 gram per kilo.

*Furfurol-sodium Bisulphite.*

The frog, when poisoned by this substance, passes gradually into a condition of paralysis: voluntary motion and reflex activity disappear, the respirations and the pulse become slower and slower and, finally, cease. The sensory nerves are somewhat depressed, but the motor nerves and muscles are apparently unaffected. The loss of reflex activity is due in part to the action on the sensory nerves, but chiefly to a depression of the spinal cord.

It may be given to dogs by the stomach, or subcutaneously, in doses as large as 1 gram per kilo. without inducing other symptoms than a decrease in the pulse, respiration and temperature. The pulse may be reduced within two hours one-third, the respirations one-half, and the temperature from 0.2°–0.8° C. When given by the stomach, vomiting is apt to ensue.

Studies by means of the kymographion show that, when injected into the vein in doses of 1 gram, the arterial pressure, pulse and respiration are diminished. Repeated doses cause further reductions, and death follows from cardiac paralysis. The failure of the pulse is due to a direct cardiac action; the fall of pressure is partly cardiac, but largely the result of a depression of the vaso-motor centres in the medulla oblongata. The respiratory changes are the result of a direct depression of the respiratory centres. Convulsions of a tetanic character were observed in one experiment, just before death.

The lethal dose by the vein is from 0.3–0.5 gram per kilo. of body-weight. 3 grams per kilo., injected subcutaneously, caused the death of a dog within twenty-four hours.

### *Oxamic Acid.*

Oxamic acid may be considered inert, since the intravenous injection of 10 grams in divided doses, in the case of a dog weighing 6.5 kilos., did not produce other effects than a slight diminution of arterial pressure and pulse; and, when given by the stomach in similar quantities, or when given to frogs, no apparent results ensue.

### *Cyanuric Acid.*

A dog weighing 14 kilos. was given 15 grams of this substance intravenously, without other effects than a slight fall of blood-pressure and a marked increase in the number of respirations.

### *Alcohols.*

Investigations made with certain of the alcohols belonging to the ethyl and aromatic series, while too few to permit of a close analysis of their individual physiological differences, enable us to reach valuable generalisations. Among the preparations which we have examined are propylic, iso-propylic, butylic, iso-butylic, heptylic, octylic, allylic, benzylic, and cuminic alcohols. From three to six experiments were performed with each, and these were confined almost solely to observations of the effects on the circulation and respiration, graphic records being made with the aid of the kymographion. In all cases a ten-per cent. solution or emulsion in water was intravenously injected.

From the results of these experiments it may be concluded that the actions of these alcohols are essentially of the same character, but differing in degree. Doubtless more detailed study will elicit some more or less distinctive peculiarities in each preparation.

Ethylic alcohol, which may be taken as a type of the ethyl series, has been carefully studied and its actions on the circulation and respiration may thus be summarised: Small doses increase the frequency and force of the heart-beat, increase the arterial pressure, and increase the respiratory movements. Large doses generally decrease, but sometimes increase, the pulse-rate; primarily increase and then lower arterial pressure, and increase the respirations. Very large doses depress pulse, pressure and respiration. The changes in the heart-beat are due to direct actions on the heart; the increase of pressure is also due to a direct action on the heart. The decrease of pressure is due to a depression both of the heart and vaso-motor apparatus. The primary

increase and final decrease in the respiration-rate are due to a stimulation and subsequent depression of the respiratory centres.

We are led to believe that the actions of the alcohols which we have examined are identical with those of ethyl alcohol, the differences being essentially in degree, and increasing with the acquisition of each radicle; in other words, the higher the alcohol in the series the greater its toxic power. Benzylic and cuminic alcohols belong to the aromatic series, and must to some extent be considered separately, although the actions, qualitatively, are apparently the same as those of the others.

These results are in accord generally with those noted by Dogiel,[1] and Dujardin-Beaumetz and Andigé.[2] Dogiel studied the five lower members of the ethyl series, and found that the actions were the same, and that the toxic power was the least in methylic and greatest in amylic alcohol. The French observers, however, found that methylic alcohol was less poisonous than ethylic alcohol, but that in passing from ethylic to amylic alcohol toxicity regularly increased. Heptylic and octylic alcohols were, when pure, about as toxic as ethylic alcohol, but when diluted with ten times their bulk of absolute alcohol the lethal doses were less than a third.

The minimal lethal doses of the alcohols examined by us when injected intravenously, although likely to be modified by more extended researches, are as follows:

| | | | | | | |
|---|---|---|---|---|---|---|
| Propylic alcohol, | 1.6 | –2.5 | cc. | per | kilo. | of body-weight. |
| Butylic " | 0.3 | –0.6 | " | " | " | " |
| Heptylic " | 0.1 | –0.12 | " | " | " | " |
| Octylic " | 1.05 | –0.1 | " | " | " | " |
| Allylic " | 0.2 | –0.24 | " | " | " | " |
| Benzylic " | 0.4 | –0.6 | " | " | " | " |
| Cuminic " | 0.25 | –0.3 | " | " | " | " |

| | | | | | | |
|---|---|---|---|---|---|---|
| Propylic alcohol, | (normal), | 1.6–2.5 | cc. | per | kilo. | of body-weight. |
| " " | Iso- | 1.3–2. | " | " | " | " |
| Butylic " | (normal), | 0.3–0.6 | " | " | " | " |
| " " | Iso- | 0.6–0.7 | " | " | " | " |

| | | | | | | |
|---|---|---|---|---|---|---|
| Butylic alcohol, | (primary), | 0.3–0.6 | cc. | per | kilo. | of body-weight. |
| " " | (secondary), | 1.0–1.5 | " | " | " | " |
| " " | (tertiary), | 1.0–1.2 | " | " | " | " |

(*To be continued.*)

[1] Pflüger's Arch. für Physiologie **8**, 605.

[2] Compt. rend. **83**, 80–82.

# ON PARA-XYLENE-DISULPHONIC ACID.[1]

By Jesse H. Holmes.

## *Introduction.*

The work to be described in this paper was undertaken in the hope of preparing a substance containing two sulphinide groups, $\begin{matrix}SO_2\\CO\end{matrix}>NH$, in the molecule, the remarkable properties of the simple sulphinide having led to the desire to learn the properties of a disulphinide $C_6H_2\left\{\begin{matrix}SO_2>NH\\CO\\SO_2>NH\\CO\end{matrix}\right.$. In order to obtain a substance of this kind, it seemed simplest to start with para-xylene and make from this a disulphonic acid, as the chances appeared to be in favor of the formation of a di-ortho acid. While a disulphonic acid of para-xylene was prepared without serious difficulty, it was not possible to obtain a disulphinide by the oxidation of its amide, and the constitution of the acid was not determined.

The acid was prepared according to the method used in this laboratory in the preparation of toluene-disulphonic acid,[2] that is, by treating xylene-sulphon-chloride with strong sulphuric acid. The details of the operation were as follows: About 35 grams of pure para-xylene, boiling at 136°–138°, were gradually added, with constant shaking, to about twice the volume of fuming sulphuric acid diluted with a small quantity of ordinary acid. The dilution was found necessary in order to avoid blackening the product; and in spite of it the sulphonic acid obtained was dark-colored. Action took place readily. In the early stages of the operation it was necessary to cool the mixture in water, and at last some warming was required to complete the reaction. After cooling the mixture, a small quantity of water was added, when the sulphonic acid crystallised out:[3] on further dilution it was redissolved. The solution was now diluted to four or five liters, the excess of sulphuric acid precipitated with chalk, the gypsum care-

[1] The work of which an account is given in this paper was undertaken at the suggestion of Professor Remsen and has been carried on under his constant supervision. It gives me pleasure to express here my appreciation of his uniform kindness and consideration.

[2] This Journal **1**, 174. [3] Ber. d. chem. Ges. **10**, 1009.

fully washed with hot water, and the solution of calcium xylene-sulphonate evaporated to about two liters. The calcium salt was now transformed into the sodium salt by the addition of a slight excess of sodium carbonate, the precipitated calcium carbonate separated by filtration, and the sodium salt solution evaporated to dryness. The sodium salt, after drying, was quite dark-colored. It was treated with an equal weight of phosphorus pentachloride, and then, as the reaction did not seem to be complete, with a considerable excess. It was found later that the yield was very unsatisfactory unless this excess was used, although every precaution was taken to have the salt perfectly dry. The action with the pentachloride took place at once, and after the evolution of hydrochloric acid ceased the semi-liquid mass was heated for some time on a water-bath, to drive off the oxychloride of phosphorus. On adding a large volume of water, xylene-sulphonchloride separated as a dark, turbid, oily liquid, which did not entirely solidify even when cooled in a freezing-mixture. According to Jacobsen,[1] this chloride, when pure, is a crystalline solid melting at 24°–26°.

The chloride was now treated with four or five times its volume of fuming sulphuric acid. Hydrochloric acid escaped at once, and, on heating, the evolution of this gas continued for some time. The heating was continued until a drop of the mixture showed no turbidity when dropped into water, this being an indication that the acid chloride was completely decomposed. The acids were now diluted and neutralised with calcium carbonate. The sodium salt was prepared as before and was treated with twice its weight of phosphorus pentachloride. After removing the phosphorus oxychloride by evaporation, the disulphon-chloride was separated in several liters of water.

*Xylene-disulphon-chloride*, $C_6H_2\begin{cases}(SO_2Cl)_2\\(CH_3)_2\end{cases}$.—In the impure state, as at first obtained, the disulphon-chloride was a tarry, brownish-yellow mass which adhered strongly to filter-paper, glass, etc. It solidifies easily when cooled in a freezing-mixture. Considerable difficulty was encountered in finding a suitable solvent from which to crystallise it. It dissolves quite readily in cold alcohol, but cannot be obtained again therefrom, probably breaking down to the corresponding disulphonic acid. It is rather easily soluble in benzene, chloroform, acetone, and glacial acetic

[1] Ber. d. chem. Ges. **10**, 1009.

acid, but does not crystallise from any of them. When the benzene solution is evaporated to dryness the chloride is left as a transparent, varnish-like cake in the bottom of the beaker, which, on standing, draws together into something like crystalline structure, still containing, of course, all the original impurities. Ordinary benzine, or "petroleum ether," was finally found to be the most satisfactory solvent. The chloride is dissolved in it by boiling in a water-bath, and the solution is allowed to cool very slowly, when the crystals separate in radiating bunches of considerable size.

After a single recrystallisation the chloride was white and appeared to be perfectly pure. The melting-point was 72°–74°. On standing in contact with the air the crystals turn slightly yellow. The substance was analysed for chlorine by the method of Carius. As preliminary experiments showed it to decompose very easily, the tube was heated only to about 100°.—

0.2652 gram of xylene-disulphon-chloride gave 0.2527 gram of silver chloride.

| | | Calculated. | Found. |
|---|---|---|---|
| $C_8H_8O_4S_2$ | 232 | 76.57 | ... |
| 2Cl | 71 | 23.43 | 23.56 |
| | 303 | 100.00 | |

An attempt was made to discover the crystal system and constants, but the planes were found to be so imperfect that the angles could not be accurately measured.

*Xylene-disulphonic Acid*, $C_6H_2(CH_3)_2(SO_3H)_2$.—The quantity of chloride prepared as just described was now divided into two parts. The larger portion was transformed into the disulphonamide, which will be spoken of later. The remainder was boiled with water for about twelve hours, in a flask connected with an inverted condenser. At the end of this time a small quantity of organic matter remained undissolved, which was not further affected by boiling water. It dissolved easily when warmed with alcohol, and apparently decomposed. The quantity was too small for any careful investigation.

The solution containing the decomposed chloride was now evaporated to dryness on a water-bath, the residue redissolved, and the evaporation repeated until no more hydrochloric acid was given off. The disulphonic acid was then dissolved again in water, and the solution was treated several times with animal

charcoal, being finally obtained clear with a slight yellow tinge. On evaporation to a small volume the acid crystallises out in needles which are exceedingly soluble in water.

*Barium Xylene-disulphonate,* $C_6H_3(CH_3)_2(SO_3)_2Ba$.—A solution of xylene-disulphonic acid was boiled with pure barium carbonate and filtered from the excess of the latter. A perfectly clear, colorless solution was obtained. On evaporation the salt is precipitated in the form of brownish scales which show some signs of crystalline structure under the microscope. It was found impossible to obtain it in good crystals, although it is rather less soluble than the other salts of the same acid. The salt contained no water of crystallisation. Analysis resulted as follows:

I. 0.2825 gram of salt gave 0.1621 gram of barium sulphate.

II. 0.2498 gram of salt gave 0.1443 gram of barium sulphate.

| | | Calculated. | Found. I. | II. |
|---|---|---|---|---|
| $C_8H_8O_6S_2$ | 264 | 65.84 | ... | ... |
| Ba | 137 | 34.16 | 33.74 | 33.97 |
| | 401 | 100.00 | | |

The analysis as well as the color showed that the substance was not entirely pure.

*Calcium Xylene-disulphonate,* $C_6H_3(CH_3)_2(SO_3)_2Ca.4H_2O$.—The calcium salt was made by neutralising the solution of free acid with finely-powdered Iceland spar. On evaporating to a small volume and allowing to stand, the salt crystallised out in tablets nearly square and with beveled edges. An approximate measurement of their angles indicated that they belonged to the triclinic system, though approaching the symmetry of the tetragonal system. The crystals are biaxial, and are cut by the plane of the optic axes very nearly or quite in the direction of the shorter diagonal.

The calcium salt is very soluble in water, dissolving easily if the solution from which it crystallises be slightly warmed. If the solution is concentrated beyond a certain point the salt is slowly decomposed and fine needle-shaped crystals of gypsum are deposited. One of the four molecules of water of crystallisation could not be driven off without decomposition of the salt. The partially dried salt, which falls apart as a fine powder, is very hygroscopic, taking up water even from calcium chloride in a

desiccator. This property seems to be common to a number of the salts of this acid, so that determinations of water are, at best, only approximate. While standing on the balance-pan, water is absorbed so rapidly as to prevent accurate determinations.

Analysis resulted as follows:

I. 0.3521 gram of the salt lost 0.0524 gram of water at 200° and gave 0.1272 gram of calcium sulphate.

II. 0.2327 gram gave 0.0840 gram of sulphate.

III. 0.1377 gram of salt lost 0.0199 gram of water and gave 0.0500 gram of sulphate.

| | | Calculated. | Found. I. | II. | III. |
|---|---|---|---|---|---|
| $C_8H_8O_6S_2H_2O$ | 282 | 75.00 | ... | ... | ... |
| $3H_2O$ | 54 | 14.36 | 14.88 | ... | 14.45 |
| Ca | 40 | 10.64 | 10.64 | 10.62 | 10.64 |
| | 376 | 100.00 | | | |

The rest of the salts of xylene-disulphonic acid were made from another stock of material prepared in the same general way, but some slight differences were introduced into the method of preparation, which are noted here because the acid obtained differed in some particulars from that previously made. The chief differences in the process were that the temperature was kept lower in both treatments with sulphuric acid than in the first case, and the transformation from xylene-sulphon-chloride to xylene-disulphonic acid was accomplished by the use of sulphuric acid of specific gravity 1.84 instead of the fuming acid. The blackening of the material was thus avoided and colorless products were obtained throughout the operations. The differences observed between the two products were not very great and may have been due to slight impurities in the first. The two chlorides show the same melting-point—about 72°—but the crystal form of the specimen last prepared was apparently different, the crystals appearing from the benzine solution as thin rectangular plates, instead of showing a prismatic habit as did the first product. The second stock of the acid itself could not be obtained in crystalline form, while the first crystallised in needles. The amide last prepared showed some slight differences, which will be mentioned when the discussion of that substance is reached. The calcium salt, which crystallised well, though not easily, in the first instance, could not be made to crystallise in the second, but sepa-

rated from the solution as an amorphous precipitate on sufficient concentration. The second lot of acid was found to contain a small quantity of sulphuric acid. It was purified by making the lead salt and decomposing its solution by action of hydrogen sulphide.

*Lead Xylene-disulphonate,* $C_6H_2(CH_3)_2(SO_3)_2Pb.3H_2O$.—The lead salt was prepared by dissolving lead carbonate in a dilute solution of the acid. It was found impossible to crystallise the salt, but it was obtained in an amorphous condition by evaporating the solution to small volume. The salt is slightly soluble in alcohol and, when this solution is evaporated nearly to dryness, it crystallises out in long, lustrous but indistinct filaments; so small a quantity goes into solution, however, that this method is not available for purification. As in the case of the crystalline calcium salt, the last molecule of water of crystallisation cannot be driven off without decomposing the compound.

The following results were obtained by analysis:

I. 0.1280 gram gave 0.0096 gram of water and 0.0742 gram of lead sulphate.

II. 0.2485 gram gave 0.1438 gram of lead sulphate.

III. 0.2156 gram lost 0.0154 gram of water.

| | | Calculated. | Found. I. | II. | III. |
|---|---|---|---|---|---|
| $C_8H_8O_6S_2.H_2O$ | 282 | 53.79 | ... | ... | ... |
| $2H_2O$ | 36 | 6.86 | 7.50 | ... | 7.14 |
| Pb | 206.4 | 39.35 | 39.52 | 39.53 | ... |
| | 524.4 | 100.00 | | | |

*Magnesium Xylene-disulphonate,* $C_6H_2(CH_3)_2(SO_3)_2Mg.7H_2O$. —This salt was prepared by dissolving magnesium oxide in a solution of the free acid. Like the other salts mentioned, it is very soluble and cannot be crystallised. It was obtained as an amorphous precipitate. As before, the water of crystallisation could not be driven off without decomposing the salt. While it is evident that, calculating the amount of water of crystallisation from the determination of the base alone is not a safe method, yet the results for the latter are so sharp that they are given for what they are worth. The attempt to drive off water was made twice and persevered in until the salt blackened. 18.74 and 18.63 per cent. of water were driven off in these two experiments, while the amount required to correspond with the amount of base present is more than 30 per cent.

I. 0.2904 gram of the salt gave 0.0853 gram of magnesium sulphate.

II. 0.2934 gram lost 0.0550 gram of water and gave 0.0861 gram of magnesium sulphate.

III. 0.1819 gram lost 0.0339 gram of water and gave 0.0525 gram of magnesium sulphate.

| | | Calculated. | Found. I. | II. | III. |
|---|---|---|---|---|---|
| $C_8H_8O_6S_2.7H_2O$ | 390 | 94.20 | ... | ... | ... |
| Mg | 24 | 5.80 | 5.87 | 5.86 | 5.87 |

The amounts of magnesium calculated for the weight of the salt after driving off as much water as possible, are as follows:

II. 0.2384 gram of dried salt gave 0.0861 gram of magnesium sulphate.

III. 0.1319 gram of salt gave 0.0535 gram of sulphate.

| | | Calculated. | Found. II. | III. |
|---|---|---|---|---|
| $C_8H_8O_6S_2$ | 264 | 91.67 | ... | ... |
| Mg | 24 | 8.33 | 7.22 | 7.19 |
| | | 100.00 | | |

*Silver Xylene-disulphonate*, $C_6H_2(CH_3)_2(SO_3Ag)_2.H_2O$.—The silver salt was prepared by dissolving pure silver oxide in a solution of the acid. After several attempts it was obtained in crystalline form as small scales with a pearly lustre. On exposure to light it blackens. Only a very small quantity was made. Analysis resulted as follows:

0.1319 gram of silver salt lost 0.0049 gram of water and yielded 0.0571 gram of silver.

| | | Calculated. | Found. |
|---|---|---|---|
| $C_8H_8O_6S_2$ | 264 | ... | ... |
| $H_2O$ | 18 | 3.62 | 3.71 |
| 2Ag | 215.3 | 43.29 | 43.29 |
| | 257.3 | | |

Discussion of the probable constitution of xylene-disulphonic acid will be given after the experiments on the oxidation of its amide have been described.

*Xylene-disulphon-amide*, $C_6H_2(CH_3)_2(SO_2NH_2)_2$.—As stated above, the main portion of xylene-disulphon-chloride was transformed into the amide. That obtained from the first preparation

will be described first, and the points of difference in the second preparation will be pointed out later.

The chloride, after being dried as completely as possible, was treated with strong ammonia, and the mixture was evaporated to dryness on the water-bath. The chloride turns yellow when first treated with the ammonia, but gradually loses this color and becomes nearly white. It was found better, after some experiments, to pass gaseous ammonia, obtained by warming its concentrated solution, over the dry chloride contained in a flask. The reaction seemed to be complete, though it was thought best to treat the amide so prepared with a small quantity of the ammonia solution before proceeding. After thorough washing with cold water the amide appeared as a fine, light-gray powder. It is very difficultly soluble in water, more easily in a mixture of alcohol and water. It dissolves readily in alkalies, and is reprecipitated by acids as a flocculent white mass. Pyridine dissolves it in large quantities, but deposits it on evaporation or dilution as an amorphous precipitate. It is only slightly soluble in glacial acetic acid, chloroform, and ether. Acetone dissolves it readily, as does also a mixture of acetone with a large proportion of water, but it cannot be crystallised from such a solution. It was finally found best to dissolve the substance in a mixture of about equal parts of alcohol and water, purify by boiling with animal charcoal, boil off most of the alcohol, and crystallise from a large volume of water. On allowing the solution in alcohol and water to cool, the disulphon-amide appears in extremely small, satiny crystals which do not settle even on long standing. When the mixture is stirred the amidê in suspension presents a very remarkable appearance, due to the varying reflections from the minute crystal surfaces. The crystals obtained from the solution in water are larger, but are still too small to be distinguished by the naked eye. As seen under the compound microscope, they are found to consist of very thin tabular plates which, in polarised light, show a complicated twinned structure. These plates show parallel extinction, and the characteristic plane angles are 60° and 30°, as nearly as they can be measured on the revolving stage of the microscope. A considerable number of different shapes were observed, the thin plates having three, four and six sides. The crystals appeared to be optically biaxial, but, on account of the extreme thinness of the plates, this could not be certainly determined.

On evaporating the mother-liquors from these crystals, a white flocculent substance was obtained which resembles the amide in some particulars, but settles more readily. A partial analysis indicated that it had the same composition, but was less pure.

An attempt was made to determine the melting-point of the disulphon-amide in a sulphuric-acid bath, but it was found not to melt below the boiling-point of the acid used. It was then determined in an air-bath made by enclosing a small test-tube in a larger one, leaving a considerable layer of air between. This device was found very convenient in determining high melting-points. The disulphon-amide melts with partial decomposition at 294°–295°.

Analysis of the crystallised substance gave the following results:

Sulphur, determined by Pearson's method:

I. 0.1882 gram gave 0.3307 gram of barium sulphate.

II. 0.1703 gram gave 0.2988 gram of barium sulphate.

Nitrogen, determined by Kjeldahl's method as modified by Gunning:

I. 0.1778 gram gave 0.0186334 gram nitrogen.

II. 0.3240 gram gave 0.0337456 gram nitrogen.

III. 0.2282 gram gave 0.0248837 gram nitrogen.

Carbon and hydrogen, determined by combustion with lead chromate and lead oxide:[1]

I. 0.2004 gram yielded 0.2687 gram of carbon dioxide and 0.0928 gram of water.

II. 0.1606 gram gave 0.2150 gram of carbon dioxide and 0.0738 gram of water.

| | | Calculated. | Found. I. | II. | III. |
|---|---|---|---|---|---|
| $C_8$ | 96 | 36.36 | 36.56 | 36.50 | ... |
| $H_{12}$ | 12 | 4.55 | 5.14 | 5.12 | ... |
| $S_2$ | 64 | 24.24 | 24.15 | 24.02 | ... |
| $N_2$ | 28 | 10.61 | 10.48 | 10.41 | 10.90 |
| $O_4$ | 64 | 24.24 | | | |
| | 264 | 100.00 | | | |

The disulphon-amide from the second stock of material possessed the same general properties as that from the first, including the melting-point. A sulphur determination after a single crystallisation indicated that it was not entirely pure. After several

[1] Method as modified by R. R. De Roode, This Journal **12**, 220.

recrystallisations some of the crystalline product was obtained, but the larger part resembled the flocculent substance obtained from the mother-liquors of the first crystallised amide. It was not determined whether these differences were due to the presence of an isomeric disulphon-amide or merely to impurities.

The oxidation of the disulphon-amide was next undertaken.—

### Oxidation of Para-xylene-disulphon-amide.

In the first experiment a very large excess of both permanganate and alkali was used. The proportions taken were:

| | |
|---|---|
| Disulphon-amide, . . . . . | 5 grams. |
| Potassium permanganate, . . . . . | 40 " |
| Potassium hydroxide, . . . . . | 10 " |
| Water, . . . . . | 1 liter. |

These were heated in a water-bath and by the introduction of a current of steam for about four hours. The excess of permanganate was then precipitated by alcohol, the filtrate from the hydroxides of manganese was brought nearly to neutrality by hydrochloric acid and was then evaporated on the water-bath to about 100 cc. On acidifying, after allowing the solution to cool, a considerable precipitation of a fine, crystalline substance occurred. This settled at once and was easily separated. After several recrystallisations it seemed perfectly pure. When heated on platinum foil it left a considerable alkaline residue, which was shown by the flame-test to contain potassium. Nitrogen also was found to be present.

After some further experiments as to the best proportions of reagents, the rest of the amide of the first stock was oxidised, using the proportion of permanganate calculated for the oxidation of the two methyl groups:

| | |
|---|---|
| Disulphon-amide, . . . . . | 20 grams. |
| Potassium permanganate, . . . . . | 48 " |
| Potassium hydroxide, . . . . . | 10 " |
| Water, . . . . . | 2½ liters. |

This solution was heated in a water-bath until it was completely decolorised. The filtrate was then concentrated *after* being acidified with hydrochloric acid. On cooling, little rosettes of white crystals appeared and, later, some of the potassium compound mentioned above.

*Disulphamine-para-toluic Acid*, $C_6H_2(CH_3)(SO_2NH_2)_2COOH.H_2O$.—The main product was repeatedly crystallised, but could not be obtained in sharp crystals. It comes out always in arborescent radiating filaments. Its melting-point, as determined in an air-jacket, was about 272°. It burns on platinum foil, leaving no residue. Titration with standard ammonia shows it to be a monobasic acid. At 105° it loses one molecule of water of crystallisation. Analysis resulted as follows:

0.1639 gram of the acid lost 0.0095 gram of water at 105°.

| | | | |
|---|---|---|---|
| $C_8H_{10}O_6S_2N_2$ | 294 | 94.23 | ... |
| $H_2O$ | 18 | 5.77 | 5.79 |
| | 312 | 100.00 | |

0.1639 gram of acid on combustion gave 0.0586 gram of water and 0.1835 gram of carbon dioxide.

0.1116 gram gave 0.0094781 gram of nitrogen.

0.1509 gram gave 0.2277 gram of barium sulphate.

| | | Calculated. | Found. |
|---|---|---|---|
| $C_8$ | 96 | 30.77 | 30.53 |
| $H_{12}$ | 12 | 3.85 | 3.97 |
| $N_2$ | 28 | 8.97 | 8.49 |
| $S_2$ | 64 | 20.51 | 20.72 |
| $O_7$ | 112 | 35.90 | ... |
| | 312 | 100.00 | |

It was thought that the potassium salt mentioned above might be the potassium salt of this acid, being precipitated by acids without decomposition in the cold because of its comparative insolubility. A small quantity was boiled with hydrochloric acid, and on cooling an insoluble acid was precipitated, which on recrystallisation resembled the acid oxidation-product, but did not melt at exactly the same point. The percentages of sulphur and nitrogen agree in indicating that the potassium compound is the salt of the acid in question with two molecules of water of crystallisation. At 150° only five-tenths of one per cent. of water was lost; but many substances containing the two sulphamine groups retain their water of crystallisation to a higher temperature. On account of lack of material more attempts to drive off the water were not made. It was supposed when the analyses were made

that all the water was driven off, a constant weight being reached. The analyses resulted as follows:

I. 0.1938 gram gave 0.0450 gram of potassium sulphate.

II. 0.1736 gram gave 0.0404 gram of potassium sulphate.

I. 0.2086 gram gave 0.0165353 gram of nitrogen.

II. 0.1630 gram gave 0.01240528 gram of nitrogen.

| | | Calculated. | Found. I. | Found. II. |
|---|---|---|---|---|
| $C_8H_9O_6S_2.2H_2O$ | 301 | 81.81 | ... | ... |
| K | 39 | 10.59 | 10.41 | 10.43 |
| $N_2$ | 28 | 7.60 | 7.92 | 7.65 |

Sulphur determinations varied so widely that they were considered of no value. It is not thought that the evidence as to the composition of this salt is at all conclusive, but it is favorable to the view stated.

*Barium Disulphamine-toluate*, $C_6H_2(CH_3)(SO_3NH_2)_2CO_2ba.2\frac{1}{2}H_2O$.—The barium salt was obtained by dissolving barium carbonate in an aqueous solution of the purified acid. The two samples analysed were made from different preparations of the acid. The salt is exceedingly soluble. It appears from the much concentrated solution in little, warty, spherical masses which are quite hard. These were dried between filters, reduced to fine powder and again pressed between filter-papers. Analysis resulted as follows:

I. 0.1539 gram of salt gave 0.0149 gram of water at 130° and 0.0442 gram of barium sulphate.

II. 0.1810 gram lost 0.0227 gram of water with slight decomposition at 240°, and gave 0.0523 gram of barium sulphate.

| | | Calculated. | Found. I. | Found. II. |
|---|---|---|---|---|
| $C_6H_9O_6N_2S_2$ | 295 | 72.21 | ... | ... |
| $2\frac{1}{2}H_2O$ | 45 | 11.02 | 9.68 | 11.15 |
| $Ba_{\frac{1}{2}}$ | 68.5 | 16.77 | 16.88 | 16.99 |
| | | 100.00 | | |

The results in this case are not satisfactory, but the composition assigned seems to be justified by the facts. In the second water determination, after the weight was taken, the crucible and salt were left in a desiccator over calcium chloride for the night. The next morning the weight showed that enough water had been

taken up to reduce the percentage removed from 11.15 to 9.45 per cent., which last is about the same quantity that remained when the salt was dried at 130°. On account of the extreme solubility of the salt it could not be purified by recrystallisation, which accounts in part for the inconclusive results as to the basic constituent.

*Calcium Disulphamine-toluate*, $C_6H_2(CH_3)(SO_2NH_2)_2CO_2ca$.—The calcium salt was prepared from finely-powdered Iceland spar. On evaporating the solution to a small volume and allowing it to cool, the whole solidifies to a stiff, transparent, jelly-like mass which could be removed from the beaker as from a mould. No attempt was made to dry it for analysis.

*Lead Disulphamine-toluate*, $C_6H_2(CH_3)(SO_2NH_2)_2CO_2pb.3H_2O$.—This salt was prepared by dissolving lead carbonate in a solution of the free acid. It crystallises out on cooling the solution in long, lustrous needles which show a tendency to arrange themselves in fan-shaped aggregates. Analysis resulted as follows:

0.1652 gram of the salt lost 0.0203 gram of water and gave 0.0551 gram of lead sulphate. There was a slight yellowing at the end of the water determination, which probably accounts for the high result.

| | | Calculated. | Found. |
|---|---|---|---|
| $C_6H_2O_6N_2S_2$ | 295 | 65.24 | ... |
| $3H_2O$ | 54 | 11.94 | 12.28 |
| $Pb_{\frac{1}{2}}$ | 103.2 | 22.82 | 22.76 |
| | 452.2 | 100.00 | |

*Silver Disulphamine-toluate*, $C_6H_2(CH_3)(SO_2NH_2)_2CO_2Ag.2H_2O$.—The silver salt was prepared by first making the neutral ammonium salt, an excess of ammonia being added to the acid solution for that purpose. The excess was driven off by evaporation and the silver salt was then precipitated by the addition of silver nitrate. It crystallises on cooling in very fine needles which have a tendency to fan-shaped arrangement, as in case of the lead salt. It is less soluble than the lead salt. A few drops of its solution added to a large volume of cold water render it milky. It decomposes with blackening on exposure to light and air, when in a moist condition. It was dried between filter-papers and analysed with the following results:

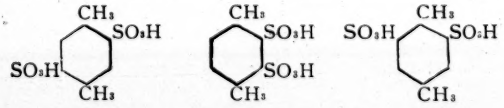
0.1563 gram lost 0.0130 gram of water at 180° and gave 0.0386 gram of silver.

| | | Calculated. | Found. |
|---|---|---|---|
| $C_8H_9O_6N_2S_2$ | 295 | 67.20 | ... |
| $2H_2O$ | 36 | 8.20 | 8.32 |
| Ag | 108 | 24.60 | 24.69 |
| | 439 | 100.00 | |

The products of oxidation of the second preparation of xylene-disulphon-amide showed the presence in small quantity of one substance not observed in the previous experiments. This consisted of crossed and radiating crystals with distinct angles. They were transparent, and differed markedly from disulphamine-toluic acid. The melting-point is about 274°. It loses no water on heating to 105°. Enough crystals were picked out for a partial analysis, which showed the presence in the compound of 10.35 per cent. of nitrogen and 24.28 per cent. of sulphur. The original disulphon-amide of which this is an oxidation-product contains 10.60 per cent. of nitrogen and 24.24 per cent. of sulphur, but does not at all resemble the substance in question. Sufficient time and material to allow a further investigation were not at my disposal.

## Conclusion.

Plainly, the evidence obtained by the oxidation of xylene-disulphon-amide, as described, is not sufficient to allow any final decision as to the constitution of the disulphonic acid in question. Since, however, in all recorded cases a methyl group and a sulphon-amide group in the ortho-relation yield a sulphinide on oxidation by permanganate, it seems probable that such a relation did not exist between the methyl group oxidised and either of the sulphon-amide groups of the disulphon-amide. There are three possible arrangements of the two sulphonic-acid groups as introduced into the molecule of para-xylene, which may be represented as follows:




In two of these, each methyl group is in the ortho-relation with a sulphonic-acid radicle. In the third, however, the two radicles occupy the two ortho-positions with reference to a single methyl group, leaving the other in the meta-position as regards both of them. Between these the evidence does not enable us to decide.

Chemical Laboratory, Johns Hopkins University.

# THE PROTEIDS OR ALBUMINOIDS OF THE OAT-KERNEL.—II.

By Thomas B. Osborne.

[*Concluded from p.* 347.]

## IV.—Proteids extracted by Water.

The water-extract of freshly-ground oats has a strong acid reaction. The acidity measured by litmus is much less than by phenol-phthalein. 100 cc. of an aqueous extract became neutral to delicate litmus-paper after adding 10 cc. of two-tenths-per cent. potash solution, and 5 cc. more of the same solution were added before phenol-phthalein showed an alkaline reaction. On neutralising the water-extract of oats with two-tenths-per cent. potash, using phenol-phthalein as an indicator, a considerable precipitate appeared, which was soluble in the slightest excess either of alkali or of the acid contained in the extract. This neutralisation-precipitate indicates the presence of so-called *acid-albumin*. The perfectly-neutralised solution when heated to boiling remained clear. On adding 10 per cent. of sodium chloride to the neutralised and filtered liquid, a considerable precipitate formed upon boiling, as well as on the addition of acetic acid. When the unneutralised extract was boiled and the resulting coagulum filtered off, neither sodium chloride nor acetic acid gave a precipitate in the filtrate on boiling. The substance remaining in solution after neutralisation, but precipitated by boiling, in the presence of sodium chloride or on the addition of acetic acid, is a *globulin*, to be described later.

The water-extract, when heated slowly in a test-tube immersed in a beaker of water which in turn was set in a larger beaker of water, showed a turbidity first at 57° C., and minute flocks appeared at 64° C. Heated to 70° and filtered, the solution remained clear until raised to boiling, when a slight precipitate formed. When 10 per cent. of sodium chloride was added to the extract, the turbidity appeared at 44° C., and flocks formed at 64° C.

Five pounds of oats were treated with 6 liters of water for twenty-four hours, pressed out, and extracted a second time for twenty-four hours with the same amount of water. The aqueous

extract reacted strongly acid to litmus, but was not neutralised, as the possible action of the acid was kept in mind, and the immediate object in view was to find what substances were extracted by the use of water alone. The two extracts were united, saturated with commercial ammonium sulphate and the precipitate thereby resulting was filtered off, and scraped from the filter, the paper was washed out with water, the solution being added to the dark olive-green precipitate which partly dissolved to a brown solution. After the solution and suspended precipitate had dialysed for fourteen days in a stream of running water, thymol being added to prevent decomposition, the solution was found to be nearly free from sulphate. The contents of the dialyser were then filtered from a dark-green precipitate which had not dissolved on removing the salts. The filtrate was found not to coagulate on boiling, and was evaporated to dryness on the water-bath, leaving a brown residue which weighed between 1 and 2 grams and gave the following reactions:

With the biuret test it yielded a red-purple color, of a bluer tint than given by peptones or albumose, which increased on standing. Millon's reagent with the aqueous solution gave a strong reaction. Alcohol of 0.9 sp. gr. dissolved a portion which, after removal of the alcohol, was readily soluble in water and reacted for proteids with Millon's reagent and the biuret test. After evaporation of the alcohol on the water-bath, the substance was but partially soluble in water. The alcoholic solution was precipitated by adding stronger alcohol. Fehling's solution gave no reaction until after heating with dilute acid, when a very slight precipitate of cuprous oxide appeared. Very dilute hydrochloric acid gave no precipitate in the solution, and stronger acid made a slight turbidity. These reactions indicate presence of a *proteose* and absence of true *albumin*.

The substance remaining after dialysing the ammonium-sulphate precipitate was treated with 10-per cent. brine of sodium chloride; the resulting solution was filtered off and dialysed till free from chlorides. Nothing precipitated on removal of the salt, the proteid being soluble in water. This fact shows that no globulin had been extracted from the substance which separated on dialysis of the aqueous extract, the globulin having been converted into an insoluble form. This dialysed solution was not coagulated by boiling, and was therefore evaporated to dryness

on the water-bath. The residue, weighing about 1 gram, was not quite completely soluble in water. As much as possible was dissolved in a small amount of water, and filtered from a slight residue. The solution was colored deep yellow-brown, contained a trace of chlorides, gave no precipitate with hydrochloric acid, either very dilute or strong. No biuret reaction was discernible in the colored solution, which gave the proteid coloration with Millon's test, and did not reduce Fehling's solution before treatment with acids. On long standing, after heating with hydrochloric acid, a little cuprous oxide separated. Evidently this substance is the same (*proteose?*) as that obtained from the solution remaining after dialysing the ammonium-sulphate precipitate.

The substance remaining after dialysis and insoluble both in water and in 10-per cent. brine, was found to be partly taken up in ½-per cent. sodium carbonate solution, and in dilute hydrochloric acid, being the result of the alteration of globulin, which had passed into solution by means of the salts derived from the oats. The neutralisation-precipitate from sodium carbonate solution was slightly soluble in 10-per cent. brine of sodium chloride. The fact that the ammonium sulphate used in this work was crude and had a slightly acid reaction and a bluish tint, to which the green color of the precipitate was due, may throw doubt on the results here recorded, but as they were mostly corroborated on repeating the trials with pure and neutral ammonium sulphate, I have given them in detail.

The results of a second examination of proteids soluble in water here follow: Five pounds of oats were treated with 7 liters of water for forty-eight hours, thrown on a sieve, the residue pressed out and treated for a short time with 6 liters more of water. The two extracts were united and allowed to settle, when the nearly-clear liquid was syphoned off and saturated with pure ammonium sulphate. After the white precipitate thus produced had stood over night, it separated in large flocks from the liquid, and was filtered out and dissolved mostly in 10-per cent. sodium chloride brine, filtered, and the clear solution dialysed. The precipitate was not completely soluble in 10-per cent. sodium chloride brine nor in water—in these respects resembling the precipitate obtained from the first extract after it had been dialysed. The clear, filtered solution in 10-per cent. sodium chloride brine, on standing, slowly deposited a part of the dissolved substance. The

solution, with the precipitate which had formed, was dialysed until free from salts, and the solution after filtering again was evaporated to dryness on the water-bath, leaving a residue of 1.11 grams. The precipitate filtered out was quite small in amount. The total proteid obtained by saturating with ammonium sulphate was less than that got in the first extraction, probably because the oats were allowed to stand twice as long in contact with water as in the case of the first extract, for it was found that the clear, filtered extract of the ammonium-sulphate precipitate in 10-per cent. sodium chloride brine, on standing, deposited the dissolved substance, so that before the conclusion of the dialysis nearly all the proteid originally soluble in water had lost its solubility.

Weyl states that water converts vegetable globulins into "albuminates," that is, into forms no longer soluble in salt-solutions but soluble in 1-per cent. sodium carbonate solution, or 0.8-per cent. hydrochloric acid, and that a long-continued action of water finally converts the albuminates thus produced into modifications that are no longer soluble in 1-per cent. sodium carbonate solution, or 0.8-per cent. hydrochloric acid. They are then not to be distinguished from "coagulated proteids." This latter change he has observed only for the globulins of peas and oats.

Transformations of this character occurred in the two extracts just described. In the first, after dialysis, the residue insoluble in 10-per cent. sodium chloride brine was partly soluble in ½-per cent. sodium carbonate solution, and in the second a body was formed insoluble in water, and in 10-per cent. sodium chloride brine, but soluble in ½-per cent. sodium carbonate solution.

To sum up—We have in the aqueous extract of the oat-kernel the following bodies:

1. An *acid-albumin*, precipitated by exact neutralisation of the extract.

2. One or more *globulins*, precipitated from the neutralised extract by sodium chloride and acetic acid; also precipitated by saturation with ammonium sulphate; remaining insoluble in water on dialysis, being, as it would appear, by the action of water, converted into an "albuminate."

3. A *proteose*, remaining in solution after dialysis of the ammonium-sulphate precipitate. This body exists also in the dialysed solution of the ammonium-sulphate precipitate from the sodium-chloride extract.

## V.—Proteids extracted by Cold Sodium Chloride Solution.

1. *Direct Extraction with Salt-solution,—Preparations* **13** *and* **14.**

Freshly-ground oats extracted with 10-per cent. brine of sodium chloride[1] at 15°–20°, gave a brown solution which, when filtered clear, coagulated as follows: Heated to 42° C., a very slight turbidity formed, which increased very slowly up to 57°, at which temperature it was still slight. Above this point it increased more rapidly, at 61° the solution being opaque, at 72° flocks formed. Heated at 73° some minutes and then filtered, the filtrate became turbid again at 70°, the turbidity increasing somewhat up to 87°; from 87° to 90° the increase was more rapid, but the amount separated at 90° was small. The solution heated to 90° and filtered, became turbid again at 85°, with little change up to 97°. Heated to boiling for a short time and filtered, the filtrate gave an abundant precipitate with acetic acid.

Dilute acetic or hydrochloric acid yields a large precipitate in the brine extract, which is insoluble in a slight excess of the acid. Either saturation with sodium chloride or dilution with water throws down a copious precipitate. The precipitate produced by water, on standing in the dilute salt solution two days, becomes insoluble in 10-per cent. sodium chloride brine and in ½-per cent. sodium carbonate solution.

Saturation with ammonium sulphate completely precipitates the proteids from this solution in 10-per cent. sodium chloride brine.

Five pounds of freshly-ground oats were treated twice with 10-per cent. sodium chloride solution, and after filtering, the extract was saturated with commercial—but perfectly neutral—ammonium sulphate, which gave a dark, greenish color to the extracted substances without otherwise affecting their properties. The bulky precipitate was filtered off, suspended in water and dialysed fourteen days, until nearly free from sulphates.

A heavy precipitate remained undissolved. This was filtered out, and the filtrate and precipitate were separately examined.

*Filtrate.*—When heated very slowly in a test-tube, in the manner previously described, the solution was found to become turbid

[1] The sodium chloride employed was the "Diamond Crystal Table Salt," Eureka Salt Company, New York, which is "remarkably free from impurities."

at 58° C.; at 70° C. flocks were formed. Heated to boiling and filtered, the filtrate gave a strong reaction with Millon's reagent. The entire solution was therefore concentrated to a small volume at 40° C., and dialysed until all salts were removed. The coagulation-point of the solution was again taken and found to be the same as before; turbidity at 58° C.; flocks at 73° C. The solution was strongly colored, appearing almost black by reflected light, and by transmitted light a greenish brown. It gave good biuret and Millon's reactions; was not affected by very dilute hydrochloric acid, but was precipitated by stronger acid in the cold. Fehling's solution suffered no reduction, either before or after the action of acid. This solution was then evaporated on a plate below 50° C., and yielded about 6 grams of a very brittle, greenish-black shining substance, very soluble in water, from which alcohol of 0.9 sp. gr. dissolved some proteid.

It is seen that when oats are extracted with 10-per cent. sodium chloride solution, a substance soluble in water and coagulating at 58° to 73° C. is formed, which does not exist in the oats originally, for the aqueous extract, when treated in the same way, yields no substance coagulable even upon boiling.

The amount of this coagulable proteid, which has the properties characteristic of an *albumin*, was too small for analysis (0.5 gram from 2½ kilos. of oats), but its importance in a study of these bodies is great, for Weyl has undertaken to classify the vegetable proteids after the manner now commonly adopted for those of animal origin, and has stated that the extracts of oats and other seeds in 10-per cent. sodium chloride solution coagulate at 55° to 60° C., and consequently contain a *myosin*, since the myosin from animal muscle coagulates under these conditions at this temperature. He also states that besides myosin, *vitellin* exists in these seeds, as shown by filtering out the coagulum formed at 60° C. and heating the solution gradually to 75°, when a second coagulation takes place.

The proteid soluble in salt-solutions, to be described further on, was found, when dissolved in a 10-per cent. sodium chloride solution, to become turbid at 81° C. and to form flocks at 97°. This then has a coagulation-point not far from that of vitellin, but it differs from that substance, in being readily precipitated by sodium chloride even before saturation.

It is seen from the experiments above described that the pres-

ence of a very little coagulable substance may be highly misleading, and gives no certain basis for classifying or identifying the vegetable proteids unless each body is separated from all the others and examined in detail by itself. Weyl did not attempt this except for one preparation from the Brazil nut (*Bertholletia*), and therefore his conclusions based on coagulation-points need further study before they can be accepted.

*Precipitate.*—The precipitate from the dialyser, filtered out of the solution just considered, was treated with 10-per cent. sodium chloride solution. A part of the substance which did not dissolve was filtered out, and the clear filtrate dialysed till free from chlorides, when the proteid was found to be precipitated. This precipitate was filtered out, washed with water, absolute alcohol and ether, and dried over sulphuric acid.

The *globulin* thus obtained, "**13**," had the following properties:

In 10-per cent. brine of sodium chloride it dissolved readily to a clear solution. Addition of an equal volume of water to this solution produced a copious precipitate. Addition of sodium chloride gave a large precipitate even before saturation. When the solution was diluted till turbid, the turbidity disappeared on warming gently.

Very dilute acetic or hydrochloric acid dissolved the substance readily when salts were not present. The addition of more acid gave no precipitate. Addition of a *very* little sodium chloride to a solution of the substance in very dilute acid, made a slight precipitate; the addition of more sodium chloride threw down an abundant curdy precipitate. The more acid present, the more salt-solution was required to produce a precipitate, and the more salt-solution present, the less the amount of acid required for precipitation.

Dilute solution of citric acid in water (1 : 2000) gave results like acetic acid and dissolved the substance readily to a solution which was *not* coagulated on boiling.

From solution of the substance in 10-per cent. brine, hydrochloric acid throws down a precipitate which is wholly insoluble even in strong solution of sodium carbonate (the filtrate giving no biuret reaction), and is also insoluble in an excess of dilute acid. Acetic acid, on the other hand, gives a precipitate which, at first, is readily soluble in *very* dilute sodium carbonate, but the solution, on standing, becomes turbid.

Hydrochloric acid, in the presence of salt, converts this pro-

teid into a "coagulated proteid"; acetic and citric acids transform it into an "albuminate."

The preparation gave the usual reactions with Millon's reagent, with cupric sulphate and potash, and with nitric acid. The solution in 10-per cent. brine of sodium chloride, on heating became turbid at 81° C., and at 97° C. the formation of flocks occurred. The same solution allowed to stand at summer temperature for more than a month showed no signs of turbidity, decomposition being prevented by adding from time to time a drop of a 20-per cent. alcoholic solution of thymol. The composition of this globulin is here given:

OAT-GLOBULIN.—*First direct Extraction with* 10*-per cent. Salt-solution*—**13.**

| | I. | II. | III. | I. Ash-free. |
|---|---|---|---|---|
| Carbon | 52.23 | 52.22 | ... | 52.32 |
| Hydrogen | 7.15 | 6.99 | ... | 7.19 |
| Nitrogen | 16.92 | 17.07 | 16.90 | 16.95 |
| Sulphur | 0.88 | ... | ... | 0.88 |
| Oxygen | ... | ... | ... | 22.66 |
| Ash | 0.20 | ... | ... | ... |
| | | | | 100.00 |

*Ash.*—0.7937 gram gave 0.0015 gram ash = 0.20 per cent.

*Carbon and hydrogen*, I.—0.3561 gram gave 0.6822 gram $CO_2$ = 52.23 per cent. C, and 0.2300 gram $H_2O$ = 7.15 per cent. H.

II.—0.4584 gram gave 0.8778 gram $CO_2$ = 52.22 per cent. C, and 0.2883 gram $H_2O$ = 6.99 per cent. H.

*Nitrogen*, I (Dumas method).—0.6225 gram gave 90.85 cc. nitrogen at 18° C. (barometer 765.5 mm. at 22° C.) = 16.92 per cent. N.

II (Kjeldahl method).—0.5760 gram gave ammonia = 11.95 cc. HCl (1 cc. = 0.00822 gram N) = 17.07 per cent. N.

III.—1.0476 gram gave ammonia = 21.54 cc. HCl = 16.90 per cent. N.

*Sulphur.*—0.8022 gram gave 0.0513 gram $BaSO_4$ = 0.00705 gram S = 0.88 per cent. S.

A second preparation of globulin was as follows: Five pounds of ground oats were digested with 8 liters of 10-per cent. sodium chloride solution for forty-eight hours, and pressed out. The residue was again subjected to the same treatment. The two

extracts were united, filtered, the clear filtrate saturated with pure ammonium sulphate, and the precipitate thus produced was filtered off. This precipitate was found to be completely soluble in 10-per cent. salt-solution, save a slight turbidity, which was readily cleared up by very dilute sodium carbonate solution. It was therefore removed from the filter, suspended in water, and dialysed for eleven days, whereby the salts were nearly all removed. The precipitate was filtered off, and the filtrate and precipitate were separately examined.

*Filtrate.*—The clear brown filtrate was evaporated to dryness at 50°, leaving an amber-brown, instead of a greenish-black, residue, as was obtained from the first salt-extract.

The properties of this residue agreed exactly with those from the first salt-extract, the difference in color being due to impurities in the ammonium sulphate used in saturating the first extract.

*Precipitate.*—The precipitate in the dialyser was found to remain almost entirely soluble in salt-solution, the trifling residue not taken up by that solvent yielded to dilute sodium carbonate, and, when this solution was neutralised with a slight excess of acetic acid, the separated substance dissolved in solution of common salt.

The dialysed precipitate was therefore filtered, washed by decantation with water, alcohol, ether, and absolute alcohol, and dried over sulphuric acid. This body had all the properties of the one similarly prepared and already described (**13**). Twenty grams of substance were thus obtained, and by extracting the oat-residue with 10-per cent. sodium chloride solution, until no more proteid was taken up, and proceeding as just detailed, ten grams more of globulin were secured. The yield was accordingly 1.3 per cent. of the air-dried oats. The analyses of this preparation here follow:

OAT-GLOBULIN.—*Second direct Extraction with* 10*-per cent. Salt-solution*—"**14**."

| | I. | II. | III. | I. Ash-free. |
|---|---|---|---|---|
| Carbon | 52.28 | 52.33 | ... | 52.37 |
| Hydrogen | 7.23 | 7.17 | ... | 7.24 |
| Nitrogen | 16.78 | 16.90 | 17.01 | 16.81 |
| Sulphur | 0.89 | ... | ... | 0.89 |
| Oxygen | ... | ... | ... | 22.69 |
| Ash | 0.21 | ... | ... | ... |
| | | | | 100.00 |

*Ash.*—0.8060 gram gave 0.0017 gram ash = 0.21 per cent.

*Carbon and hydrogen*, I.—0.2894 gram gave 0.5545 gram $CO_2$ = 52.28 per cent. C, and 0.1883 gram $H_2O$ = 7.23 per cent. H.

II.—0.2190 gram gave 0.4200 gram $CO_2$ = 52.33 per cent. C, and 0.1412 gram $H_2O$ = 7.17 per cent. H.

*Nitrogen*, I (Dumas method).—0.5314 gram gave 77.1 cc. nitrogen at 18° C. (barometer 763.5 mm. at 22° C.) = 16.78 per cent. N.

II (Kjeldahl method).—0.4732 gram gave ammonia = 9.73 cc. HCl (1 cc. = 0.00822 gram nitrogen) = 16.90 per cent. N.

III.—0.9973 gram gave ammonia = 20.64 cc. HCl = 17.01 per cent. N.

*Sulphur.*—0.8400 gram gave 0.0547 gram $BaSO_4$ = 0.007513 gram S = 0.89 per cent. S.

2. *Extraction with Salt-solution after treatment with Alcohol,—Preparation* "**15**."

Since digestion of oats first with water or with salt-solution altered the proteid soluble in alcohol, it was thought possible that by preliminary treatment with alcohol a body might be obtained soluble in salt-solution and yet different from the one extracted directly by that solvent.

To test this hypothesis, three pounds of fine-ground oats were three times extracted, in the cold, with 6 liters of alcohol of 0.9 sp. gr. After pressing out as thoroughly as possible, the residue was treated three times with 10-per cent. sodium chloride solution, the solvent each time being allowed to stand in contact with the oats for twenty-four hours. The first and second extracts were united, filtered, and saturated with sodium chloride. The precipitate was filtered out and found to be but partly soluble in 10-per cent. brine. It was therefore dissolved in solution of sodium carbonate of ½ per cent., and the filtered liquor was exactly neutralised with acetic acid, which separated the proteid as a white precipitate. After washing thoroughly, by decantation with distilled water, with dilute alcohol, with ether, and finally with absolute alcohol, the substance was dried over sulphuric acid. As it weighed only 2.1 grams, the greater part of the proteid in the oats, soluble in salt-solution, had evidently been converted into an "albuminate."

OAT-GLOBULIN.—*Extracted by 10-per cent. Salt-solution after treatment with Alcohol; dissolved in ½-per cent. Sodium Carbonate Solution, and precipitated by Acetic Acid*—**15**.

| | | Ash-free. |
|---|---|---|
| Carbon | 52.39 | 52.48 |
| Hydrogen | 6.93 | 6.94 |
| Nitrogen | 16.82 | 16.85 |
| Sulphur | 0.57 | 0.57 |
| Oxygen | ... | 23.16 |
| Ash | 0.17 | ... |
| | | 100.00 |

*Ash.*—0.3773 gram gave 0.0006 gram ash = 0.17 per cent.

*Carbon and hydrogen.*—0.3187 gram gave 0.6122 gram $CO_2$ = 52.39 per cent. C, and 0.1993 gram $H_2O$ = 6.93 per cent. H.

*Nitrogen* (Dumas method).—0.3220 gram gave 47.17 cc. nitrogen at 18° C. (barometer 759.3 mm. at 28° C.) = 16.82 per cent. N.

*Sulphur.*—0.7435 gram gave 0.0304 $BaSO_4$ = 0.004216 gram S = 0.57 per cent. S.

## VI.—PROTEIDS EXTRACTED BY WEAK POTASH SOLUTION.

### 1. *Extraction after treatment of the Ground Oats with Alcohol,*—*Preparation* **16**.

Another extraction was made in nearly the same manner as that employed by Kreusler in preparing his "Oat-Legumin": Five pounds of freshly-ground oats were treated in the cold with alcohol of 0.9 sp. gr. as long as any proteid was removed. The residue was digested for some time with 7 liters of $\frac{2}{10}$-per cent. potash solution. The whole was then thrown on a sieve and the turbid percolate allowed to stand for twenty-four hours.

The dark-colored solution was syphoned off, let stand another day, decanted from the slight sediment, and then precipitated with very dilute acetic acid. After a day the substance had so far settled that about two-thirds of the liquid could be decanted. To the remainder, alcohol was added until the solution had a sp. gr. of 0.93. On further standing, the precipitate deposited and was transferred to a filter. It was washed with stronger alcohol, removed from the paper and dissolved again in $\frac{2}{10}$-per cent.

potash solution. A turbid liquid resulted which could not be filtered until a little sodium chloride had been added, which precipitated some of the dissolved proteid. This precipitate was soluble both in additional potash solution and in brine. More salt was therefore added, the nearly-clear solution was then filtered, which process lasted for about a week. Decomposition was entirely prevented by the use of thymol. The filtrate obtained each day was precipitated with dilute acetic acid, the precipitate was washed on a filter with water, then with dilute and afterwards with strong alcohol, and was finally transferred to a flask filled with absolute alcohol. When the filtration was completed and the united precipitates had been digested with absolute alcohol, they were treated with ether and again with absolute alcohol, and lastly dried over sulphuric acid, yielding preparation "**16.**"

OAT-PROTEID.—*Extracted by $\frac{2}{10}$-per cent. Potash Solution after treatment of the Ground Oats with Alcohol*—**16.**

| | I. | II. | III. | I. Ash-free. |
|---|---|---|---|---|
| Carbon | 52.11 | 52.18 | ... | 52.45 |
| Hydrogen | 6.87 | 6.74 | ... | 6.92 |
| Nitrogen | 16.52 | 16.48 | 16.40 | 16.63 |
| Oxygen | ... | ... | ... | 23.19 |
| Sulphur | 0.81 | ... | ... | 0.81 |
| Ash | 0.66 | ... | ... | ... |
| | | | | 100.00 |

*Ash.*—0.7373 gram gave 0.0049 gram ash = 0.66 per cent.

*Carbon and hydrogen*, I.—0.3666 gram gave 0.7005 gram $CO_2$ = 52.11 per cent. C, and 0.2265 gram $H_2O$ = 6.87 per cent. H.

II.—0.4523 gram gave 0.8651 gram of $CO_2$ = 52.18 per cent. C, and 0.2746 gram $H_2O$ = 6.74 per cent. H.

*Nitrogen*, I (Dumas method).—0.3577 gram gave 51.44 cc. nitrogen at 18° C. (barometer 758.7 mm. at 22° C.) = 16.52 per cent. N.

II (Kjeldahl method).—0.5037 gram gave ammonia = 10.1 cc. HCl (1 cc. HCl = 0.00822 gram N) = 16.48 per cent. N.

III.—1.0412 gram gave ammonia = 20.77 cc. HCl = 16.40 per cent. N.

*Sulphur.*—0.8670 gram gave 0.0510 gram $BaSO_4$ = 0.0070 S = 0.81 per cent. S.

For comparison the analyses of the last four preparations are here stated together:

| | **13.** | **14.** | **15.** | **16.** |
|---|---|---|---|---|
| Carbon | 52.32 | 52.37 | 52.48 | 52.45 |
| Hydrogen | 7.19 | 7.24 | 6.94 | 6.92 |
| Nitrogen | 16.95 | 16.81 | 16.85 | 16.63 |
| Sulphur | 0.88 | 0.89 | 0.57 | 0.81 |
| Oxygen | 22.66 | 22.69 | 23.12 | 23.19 |
| | 100.00 | 100.00 | 100.00 | 100.00 |

**13** and **14**, extracted directly by 10-per cent. salt-solution.

**15**, extracted by 10-per cent. salt-solution after treating the ground oats with alcohol, then dissolved in ½-per cent. sodium carbonate solution and precipitated by acetic acid.

**16**, extracted by $\frac{2}{10}$-per cent. potash solution after completely exhausting the ground oats with alcohol.

The four preparations agree in composition so closely that it is fair to assume that they were pure and that the analyses correctly represent the composition of one and the same proteid.

Of the above analyses, **13** and **14** represent the composition of oat-globulin as extracted by 10-per cent. solution of pure sodium chloride and still soluble in that menstruum, while **15** and **16** represent the insoluble or "albuminate" modification of the same proteid.

Analysis of **15** indicates that the globulin is not essentially changed in composition by being dissolved in weak sodium carbonate solution and precipitated therefrom by dilute acid.[1]

Since all proteids, except the coagulated, are soluble in weak alkali, the analysis of **16**, made on a substance extracted by $\frac{2}{10}$-per cent. potash solution, may be taken to exhibit the composition of whatever uncoagulated proteids remain in the oat after the direct extraction with weak alcohol. The close agreement of the analysis of **16** with the other analyses leads to the conclusion that the oat-proteids which are not extracted by weak alcohol mainly consist of either the globulin soluble in salt-solution or its "albuminate" modification, or else of a proteid from which these are derived under the influence of the solvents employed in their preparation.

[1] The low figure for sulphur in **15** is accidental, some loss having been incurred in oxidising the substance by fusion with alkali nitrate and carbonate. The determination could not be repeated for lack of material.

The amount of these preparations obtained in a form suitable for analysis was a very small fraction of the total proteid contained in the oats. This was probably due to the conversion of the globulin into an "albuminate" or a "coagulated proteid."

The exact cause of this change has not been ascertained. To test the hypothesis that it might be due to the action of an acid or acid salt contained in the oats, 100 grams of the freshly-ground grain were treated with 800 cc. of $\frac{2}{10}$-per cent. potash solution. The resulting mixture was neutral to litmus and did not redden phenol-phthalein. Eighty grams of sodium chloride were then added, and the solution, after standing some time, was filtered. Very little proteid was thus extracted. In another trial, 10-per cent. solution of sodium chloride mixed with $\frac{2}{10}$-per cent. of sodium carbonate, extracted but a small part of the proteids.

100 grams of oats treated with $\frac{2}{10}$-per cent. solution of sodium carbonate gave an extract containing much proteid, which on addition of a little sodium chloride yielded an abundant precipitate. Neutralising the sodium carbonate extract gave a precipitate which was insoluble in sodium chloride solution.

These experiments indicate that the insolubility of the greater part of the oat-proteids in 10-per cent. brine is not due to the presence of an acid or acid salt.

Weyl has stated that when wheat-flour is extracted with sodium chloride solution, the residue yields no gluten on kneading with water, and he considers that the gluten does not pre-exist in the wheat but is a product of ferment action. It was thought that if a ferment occasioned alteration of oat-proteids, its effect might perhaps be prevented by treating the ground oats with a sodium chloride solution heated to 75° C., so that all the soluble proteids coagulable below that temperature would be rendered insoluble and inoperative as ferments. The globulin coagulating at 80° C. ought not to be affected by this treatment. Experiments made to prove this view did not result in the extraction of much if any more globulin than was obtained when a cold salt-solution was employed.

The trials next to be described have an important bearing on the alterations of proteids which take place in the presence of water.

2. *Direct Extraction of the Ground Oats with weak Potash Solution,—Preparation* "**17**."

100 grams of freshly-ground oats were treated with 500 cc. of $\frac{2}{10}$-per cent. potash solution. The mixture, after standing some time, was found to be neutral to litmus-paper. It was then strained through a coarse cloth to remove the husks, and the residue was treated with 100 cc. of $\frac{2}{10}$-per cent. potash solution and squeezed out nearly dry. The solutions and washings were united and 100 cc. $\frac{2}{10}$-per cent. potash solution added thereto, making 700 cc. in all. The liquid was then faintly alkaline to litmus. On standing, the insoluble matter settled out; the solution was decanted, filtered, and the residue treated again with $\frac{2}{10}$-per cent. potash solution. The first potash-extract was very dark brown in color, the second much lighter. A third extract contained very little proteid. The first and second extracts were united, filtered and precipitated with acetic acid added to acid reaction, washed thoroughly with water, alcohol and ether, and dried over sulphuric acid. The preparation weighed 7.8 grams. A portion treated with $\frac{2}{10}$-per cent. potash-lye was completely soluble, giving a perfectly clear solution. The rest of the preparation was treated with hot alcohol of 0.9 sp. gr. which took up a little proteid. It was washed with hot alcohol of 0.9 sp. gr. until nothing more was removed, then with absolute alcohol and finally with ether, and was dried over sulphuric acid. After drying at 110° C. the substance was analysed with the following results:

OAT-PROTEID.—*Directly extracted with* $\frac{2}{10}$*-per cent. Potash Solution*—**17**.

| | I. | II. | Ash-free I. | Ash-free II. |
|---|---|---|---|---|
| Carbon | 52.96 | ... | 53.49 | ... |
| Hydrogen | 6.93 | ... | 7.01 | ... |
| Nitrogen | 16.23 | 16.10 | 16.39 | 16.26 |
| Sulphur | 0.98 | ... | 0.99 | ... |
| Oxygen | ... | ... | 22.12 | ... |
| Ash | 1.00 | ... | ... | ... |
| | | | 100.00 | |

*Ash.*—0.7063 gram, dried at 110°, gave 0.0071 gram of ash = 1.00 per cent.

*Carbon and hydrogen.*—0.2651 gram gave 0.5148 gram $CO_2$ = 52.96 per cent. C, and 0.1654 gram $H_2O$ = 6.93 per cent. H.

*Nitrogen,* I (Kjeldahl method).—0.7395 gram gave ammonia = 14.6 cc. HCl (1 cc. = 0.00822 gram N) = 16.23 per cent. N.

II (Dumas method).—0.4285 gram gave 59.82 cc. nitrogen at 15° C. (barometer 751.5 mm. at 21° C.) = 16.10 per cent. N.

*Sulphur.*—0.5196 gram gave 0.0373 gram $BaSO_4$ = 0.00512 gram S = 0.98 per cent. S.

3. *Extraction of the Ground Oats with weak Potash solution after one hour's treatment with Water,—Preparation* "**18.**"

One hundred grams of ground oats were next treated with 800 cc. of water, the mixture was passed through a cloth to remove husks, and the residue washed with 200 cc. of water. The liquid, with suspended starch, etc., was made up to the volume of a liter, and, after the oats had been for an hour in contact with the water, an equal volume of $\frac{2}{10}$-per cent. potash solution was added and intermixed.

After standing over night the strongly-colored solution was decanted from the residue, filtered, precipitated with acetic acid, the precipitate washed with hot alcohol until nothing more could be removed, and then with absolute alcohol and ether, and dried over sulphuric acid. This preparation weighed 4.25 grams. The residue was treated a second time with $\frac{2}{10}$-per cent. solution of potash and *very little* more proteid was extracted. After drying at 110° C. this preparation was analysed with the following results:

OAT-PROTEID.—*Extracted by* $\frac{2}{10}$*-per cent. Potash solution after one hour's contact of the Ground Oats with Water*—**18.**

| | | | Ash-free. | |
|---|---|---|---|---|
| | I. | II. | I. | II. |
| Carbon | 51.51 | ... | 52.36 | ... |
| Hydrogen | 7.16 | ... | 7.27 | ... |
| Nitrogen | 16.95 | 17.03 | 17.23 | 17.31 |
| Sulphur | 0.69 | ... | 0.70 | |
| Oxygen | ... | ... | 22.44 | |
| Ash | 1.63 | ... | ... | |
| | | | 100.00 | |

*Ash.*—0.2760 gram, dried at 110° C., gave 0.0045 gram ash = 1.63 per cent.

*Carbon and hydrogen.*—0.2953 gram gave 0.5576 gram $CO_2$ = 51.51 per cent. C, and 0.1904 gram $H_2O$ = 7.16 per cent. H.

*Nitrogen*, I (Kjeldahl method).—0.5867 gram gave ammonia = 12.1 cc. HCl (1 cc. = 0.00822 gram N) = 16.95 per cent. N.

II (Dumas method).—0.5120 gram gave 75.49 cc. N at 15° C. (barometer 753.6 mm. at 23° C.) = 17.03 per cent. N.

*Sulphur.*—0.7210 gram gave 0.0365 gram $BaSO_4$ = 0.005013 S = 0.69 per cent. S.

In this analysis the fusible ash made the combustion of the last traces of carbon difficult; hence, probably ash and nitrogen are stated a little too high, and the carbon is slightly inaccurate.

4. *Extraction with Potash solution after a day's contact of the Ground Oats with Water,—Preparation* "**19**."

100 grams of oats were treated in the manner just described, except that instead of the 1000 cc. of potash solution first added, 1000 cc. of distilled water were used. After standing a day, the aqueous solution, which had become clear, was decanted and 1000 cc. of $\frac{2}{10}$-per cent. potash solution were added to the residue and let stand upon it over-night. The solution was then decanted from the residue, filtered, precipitated with acetic acid, the precipitate was washed with hot alcohol of 0.9 sp. gr. and with absolute alcohol and ether, and dried over sulphuric acid. The preparation weighed 2.55 grams. The following figures were obtained in its analysis:

OAT-PROTEID.—*Extracted by $\frac{2}{10}$-per cent. Potash solution after one day's contact with Water*—**19.**

| | | Ash-free. |
|---|---|---|
| Carbon | 52.29 | 52.61 |
| Hydrogen | 6.88 | 6.92 |
| Nitrogen | 16.89 | 16.99 |
| Sulphur | 0.89 | 0.89 |
| Oxygen | 22.46 | 22.59 |
| Ash | 0.59 | ... |
| | 100.00 | 100.00 |

*Ash.*—0.5578 gram, dried at 110° C., gave 0.0033 gram ash = 0.59 per cent.

*Carbon and hydrogen.*—0.3300 gram gave 0.6327 gram $CO_2$ = 52.29 per cent. C, and 0.2042 gram $H_2O$ = 6.88 per cent. H.

*Nitrogen* (Kjeldahl method).—0.5426 gram gave ammonia = 11.15 cc. HCl (1 cc. = 0.00822 gram N) = 16.89 per cent. N.

*Sulphur.*—0.8310 gram gave 0.0543 gram $BaSO_4$ = 0.00746 gram S = 0.89 per cent. S.

The three preparations last described were made by extraction with the same dilute potash solution, and under essentially the same conditions in all respects, with the single difference, that while in case of **17** the oats were directly treated with this solvent, **18** was extracted by potash solution after the ground oats had been kept in contact with water for one hour, and **19** after digestion with water for twenty-four hours.

The amount of proteid extracted in the first instance was 7.80 grams.—**17.**

The amount of proteid extracted in the second instance was 4.25 grams.—**18.**

The amount of proteid extracted in the third instance was 2.55 grams.—**19.**

The composition of the three preparations is placed in comparison with that of the oat-globulin **13,** in the subjoined statement.

OAT-PROTEID.

| | Extracted by— | | | |
|---|---|---|---|---|
| | Salt-solution. | Potash Solution— | | |
| | Direct. | Direct. | After contact with water for 1 hour. | After contact with water for 24 hours. |
| | **13.** | **17.** | **18.** | **19.** |
| Carbon | 52.32 | 53.49 | 52.36 | 52.61 |
| Hydrogen | 7.19 | 7.01 | 7.27 | 6.92 |
| Nitrogen | 16.95 | 16.39 | 17.23 | 16.99 |
| Sulphur | 0.88 | 0.99 | 0.70 | 0.89 |
| Oxygen | 22.66 | 22.12 | 22.44 | 22.59 |
| | 100.00 | 100.00 | 100.00 | 100.00 |

The experiments just detailed tend to show that the action of water upon the crushed oat-kernel induces rapid changes of composition in these proteids simultaneously with their conversion into insoluble modifications.

They also indicate that the *globulin* itself is a result of changes brought about in the presence of water, and is not a body existing as such, ready-formed, in the oats.

### 5. *Norton's Avenine and Kreusler's Oat-Legumin.*

The substance extracted directly by potash solution without previous exposure of the oats to water, **17**, is probably the same as that which Johnston and Norton originally termed *avenine*, and which the latter obtained by digesting ground oats in water to which a little ammonia was added to prevent souring, as the weather was very warm.[1] The preparation which Norton analysed as avenine was, however, extracted by water alone, and was accordingly a mixture.[2] Both Norton and Kreusler applied weak alkali solution to the extraction of proteid bodies from oats *after* the ground grain had been subjected to the action of water.

Norton made and analysed two preparations, accomplishing the extraction by means of dilute ammonia, and threw down the proteid by adding acetic acid to the ammoniacal solution. One preparation, "**A**," was redissolved in strong ammonia at nearly the boiling-point, and again precipitated by acetic acid, then washed (presumably with water), and boiled in alcohol and ether until no more fatty matter was taken up. Another preparation, "**B**," was boiled in alcohol and ether *before* redissolving in very dilute ammonia at 70°–80° C.

Norton made a single analysis of A and two fairly-accordant analyses of B, the average of which is the basis for the corrected analysis given below. He found in both substances 1.00 per cent. of phosphorus, which he includes in the analyses reckoned ash-free. This phosphorus in A nearly equals, and in B exceeds, the ash. If this phosphorus be deducted and the analyses recalculated to a percentage-statement we have the corrected analyses given below. As Norton published no analytical details and used a method of doubtful accuracy (Berthier's) for estimating phosphorus, this correction is perhaps not altogether satisfactory. Norton's nitrogen determinations were all made by Dumas' method. He gives to this substance no special name, but designates it as a "protein body" simply.

Kreusler's preparation of "*Oat-legumin* **A**" was obtained by suspending 1½ pounds crushed oats in 5 liters of water, adding, to neutralise the acidity, over 3 grams of potassium hydroxide. The mixture stood twelve hours in a cool place, was then thrown on a sieve, the residue washed with water and the liquid left to rest over-night. The turbid liquid was poured off and acidified

[1] Am. J. Sci. [2] 5, 23.

[2] Loc. cit. p. 28.

with acetic acid. The precipitate was washed first with alcohol of 40 per cent., then with that of 80 per cent., then with ether, and lastly was treated with absolute alcohol and dried over sulphuric acid and at 100°. To purify this substance from starch it was dissolved in dilute potash (1 gram to the liter of water), and, from the solution after deposition of all suspended matter, it was thrown down again by acetic acid, washed and dried.

Another preparation, "**B**," was obtained from oats that had been extracted by cold alcohol—and a third, "**C**," from oats exhausted with hot alcohol—previous to treatment with weak potash solution. The analyses of **A** and **C**, corrected by deduction of ash and phosphoric acid, are here tabulated. In his preparation B, Kreusler found 16.74 per cent. of nitrogen, but carried the analysis no further.

PROTEID *obtained from Ground Oats after treatment with Water by extracting with weak solution of—*

| | *Ammonia.* (Norton.) | | *Potash.* (Kreusler.) | |
|---|---|---|---|---|
| | **A.** | **B.** | **A.** | **C.** |
| Carbon | 53.72 | 52.35 | 52.09 | 51.58 |
| Hydrogen | 7.00 | 6.93 | 8.03 | 8.01 |
| Nitrogen | 16.94 | 16.55 | 16.83 | 17.61 |
| Sulphur | 0.60 | 1.12 | 0.96 | ... |
| Oxygen | 21.74 | 23.05 | 22.09 | ... |
| | 100.00 | 100.00 | 100.00 | |

Norton remarks of his preparation **A** that, dissolving it in hot strong ammonia would seem to have darkened the color and somewhat affected the composition. It will be observed that Norton's **B** does not differ greatly from my analyses **13** and **19**.

Kreusler, doubting the purity of his preparations, boiled them in alcohol of 60 per cent., and finding that something was thereby dissolved, especially from **A**, continued this treatment as long as anything was removed. The dissolved substance contained 16.38 per cent. of nitrogen. The thus "purified" preparations had the following composition, ash-free:

| | **A.** | **C.** | Average. |
|---|---|---|---|
| Carbon | 51.40 | 51.85 | 51.63 |
| Hydrogen | 7.49 | 7.49 | 7.49 |
| Nitrogen | 17.29 | 17.03 | 17.16 |
| Sulphur | 0.85 | 0.73 | 0.79 |
| Oxygen | 22.97 | 22.90 | 22.93 |
| | 100.00 | 100.00 | 100.00 |

The average of the last two analyses Kreusler gives as expressing the composition of OAT-LEGUMIN.[1] He observes, in concluding his paper, that the substance, purified by long boiling with alcohol, had almost entirely lost solubility both in potash and acetic acid. It was in fact converted into coagulated proteid; but, that the change of composition was not entirely due to the removal of impurities and in part at least was owing to alteration of the substance itself, must be regarded as almost certain.

It is to be concluded that the "globulin" extracted by 10-per cent. salt-solution, and the "legumin" removed by dilute potash solution after action of water upon the crushed grain, are very similar, perhaps identical in composition, and that neither of them pre-exists in the grain; but the investigation on these points is not altogether conclusive and needs to be continued.

Assuming that Kreusler's analyses are correct, there can be little doubt that his oat-legumin as finally analysed was different from the proteid at first existing in the potash-extract, and was essentially altered in composition as well as in solubility during the processes of preparation and "purification."

### VII.—PROTEID EXTRACTED BY HOT SODIUM CHLORIDE SOLUTION,—Preparation "20."

Freshly-ground oats were treated with distilled water heated to 65° C., and then sufficient sodium chloride was added to make a 10-per cent. solution. When the addition of salt was begun the liquid had a temperature of 60° C.; when finished, the temperature was 45°. The mixture was digested for about an hour at 45° and a portion of the extract was filtered. The filtrate became turbid immediately on cooling. When gently warmed the turbidity completely disappeared. The perfectly clear solution on further heating coagulated as follows: at 57° C. a very slight turbidity was seen that increased but little up to 78°, whence it rapidly augmented with formation of flocks at 85°.

The remainder of the extract, together with the crushed oats,

[1] In a paper already referred to (Pflüger's Archiv **18**, 236), Ritthausen decided that the soda-lime method used by Kreusler was untrustworthy, and stated, as the result of his own analysis by Dumas' method, the nitrogen-content of *Oat-legumin* to be 17.45 per cent. It is now well understood that Dumas' method, carried out as Ritthausen describes in the paper referred to, usually gives too high results, due to retention of air in the oxide of copper, etc., and formation of carbon monoxide during the combustion.—See Report Conn. Station, 1878, p. 111; and 1879, p. 124.

was heated up to 75° and filtered as rapidly as possible. A perfectly clear filtrate was obtained which became turbid on cooling. The vessel containing the hot extract was placed in a large water-bath at 75° and cooled very slowly. A dense deposit formed which closely adhered to the bottom of the dish, and under the microscope was seen to consist entirely of minute *spheroids* about 0.01 mm. in diameter.

The residual oats, extracted twice again, yielded little more to the hot salt-solution. The proportion of proteid thus obtained was apparently little, if any, greater than that dissolved by sodium chloride solution at 15° to 20°.

The deposit of spheroids was almost wholly freed from the mother-liquor by simple decantation, and was treated with cold 10-per cent. sodium chloride solution, in which it dissolved very slowly. On warming to about 40° the substance melted to a soft, plastic mass, which became softer as the temperature rose, and could be drawn out into glistening threads. At 65° the substance became so fluid that the mass broke up and rapidly dissolved to a clear solution.

When a portion of this solution was heated to 78° a slight turbidity developed that increased very little up to 98°, at which point a few flocks separated. These were filtered out, and the filtrate gave on boiling a considerable coagulum. The filtrate from this coagulum yielded another precipitate on boiling again, and the same result followed these operations to the fourth time. The final filtrate gave with hydrochloric acid a copious precipitate.

This substance was now prepared in greater quantity in the following manner: Five pounds of ground oats were treated with twelve liters of 10-per cent. sodium chloride solution, strained through a hair-sieve to remove the husks, and then heated to 60° in a water-bath of 70° and maintained at that temperature for an hour. The extract was then filtered as rapidly as possible. The extract, after decanting from a deposit which separated out on cooling, was saturated with pure ammonium sulphate, and the precipitate thus formed was filtered off and added to the deposit formed on cooling. The substance thus obtained was then treated with three liters of 10-per cent. sodium chloride solution heated to 65° in a water-bath of 70° and filtered as rapidly as possible on a funnel surrounded by hot water. The clear filtrate, which became

turbid at once on cooling, was received in a vessel set in a large water-bath heated to 70°, and the whole was allowed to cool very slowly. When cold the liquid was decanted from the dense deposit that adhered to the bottom and sides of the beaker.

As the substance dissolved to an opalescent solution in distilled water, it was washed with 50-per cent. alcohol as long as with silver nitrate any chlorine was discoverable in the washings; then the washing was continued with absolute alcohol and with ether, and the substance dried over sulphuric acid: 8.5 grams of substance were thus obtained. The salt solution from which the spheroids had deposited was next dialysed, and the proteid which separated out on the removal of the salts was filtered off, washed in succession with dilute alcohol, absolute alcohol, and ether, and dried over sulphuric acid. This preparation weighed 5.64 grams.

The residue of the oats was extracted a second time in the same manner, and the solution was at once saturated with ammonium sulphate. The precipitate thus produced was washed directly with 50-per cent. alcohol, but since the substance dissolved on washing with dilute alcohol as long as much ammonium sulphate remained, the final yield was only 2.6 grams.

The product obtained from the solution of the first ammonium sulphate precipitate in hot brine has the following properties: Under the microscope, before washing, it appears to be perfectly homogeneous and to consist entirely of spheroids about 0.01 mm. in diameter. After washing and drying it forms a dense, snow-white powder. In cold distilled water it dissolves to an opalescent solution which gives a heavy precipitate on the addition of a little sodium chloride; a little more sodium chloride precipitates the aqueous solution almost completely, and on addition of still more salt this precipitate is again dissolved.

Hot distilled water dissolves this proteid completely to a perfectly clear solution from which a considerable part of the substance deposits on cooling.

Dilute acetic acid alone gives no precipitate in the aqueous solution; addition of a little salt together with the acid precipitates it. The precipitate thus produced is soluble in alcohol of 0.9 sp. gr.

When treated with hot dilute alcohol the substance melts and remains suspended in the solution in transparent droplets; addition of either a little salt or a little acetic acid produces no change,

but both together give a clear solution. This solution in alcohol, on cooling, forms a very bulky, transparent jelly. On evaporation, the substance separates as a skin on the surface, which is readily soluble again in dilute alcohol of 0.9 sp. gr.

On analysis the composition of the dried spheroids was found to be as follows:

OAT-PROTEID.—*Extracted by Sodium Chloride Solution at 65° C.*—**20.**

| | | | Ash-free. | |
|---|---|---|---|---|
| | I. | II. | I. | II. |
| Carbon | 52.13 | 52.16 | 52.20 | 52.24 |
| Hydrogen | 6.93 | 7.02 | 6.94 | 7.03 |
| Nitrogen | 17.72 | 17.87 | 17.75 | 17.90 |
| Sulphur | 0.81 | 0.73 | 0.81 | 0.73 |
| Oxygen | ... | ... | 22.30 | 22.10 |
| Ash | 0.19 | ... | ... | ... |
| | | | 100.00 | 100.00 |

*Ash.*—0.4694 gram substance, dried at 110° C., gave 0.0009 gram of ash = 0.19 per cent.

*Carbon and hydrogen*, I.—0.3639 gram dried substance gave 0.6955 gram $CO_2$ = 52.13 per cent. C, and 0.2270 gram $H_2O$ = 6.93 per cent. H.

II.—0.4728 gram dried substance gave 0.9036 gram $CO_2$ = 52.16 per cent. C, and 0.2985 gram $H_2O$ = 7.02 per cent. H.

*Nitrogen*, I (Kjeldahl method).—0.7768 gram substance gave ammonia = 16.75 cc. HCl Sol. (1 cc. = 0.00822 gram N) = 17.72 per cent. N.

II (Dumas method).—0.4080 gram dried substance gave 62.36 cc. N at 15° C. (barometer 762 mm. at 21° C.) = 17.87 per cent. N.

*Sulphur*, I.—0.7841 gram dried substance gave 0.0463 gram $BaSO_4$ = 0.0064 gram S = 0.81 per cent. S.

II.—0.8670 gram dried substance gave 0.0460 gram $BaSO_4$ = 0.0063 gram S = 0.73 per cent. S.

The fact that this substance separated from warm sodium chloride solutions so readily in the form of spheroids, indicated that, under proper conditions, it might be obtained in recognisable crystals. After a very large number of attempts which resulted only in the production of spheroids, a portion of that

substance which had deposited on dialysing the mother-liquor from **20**, was converted into a mixture of spheroids and crystals. The latter were about 0.1 mm. in their greatest diameters and to all appearance had distinct rhombic faces, but they were not perfectly developed and have not since been observed, many efforts to reproduce them having completely failed.

These crystals were obtained by saturating with the globulin a 10-per cent. sodium chloride solution heated to about 60° C., and allowing it to cool slowly in a large bath of warm water.

After many trials with Preparation **20** a portion of it was completely converted into perfectly-formed octahedral crystals which Prof. S. L. Penfield kindly examined and pronounced to be isometric. These crystals were obtained by dissolving some of the substance in cold distilled water and cautiously adding sodium chloride until a copious precipitate resulted. On immersing the test-tube in warm water the precipitate dissolved to a perfectly clear solution. This solution was then allowed to cool slowly in a bath containing about four liters of water heated to 60° C. After a day the deposit was examined under the microscope and found to consist entirely of crystals.

By saturating *distilled water* heated to 60° C. with Preparation **20** and allowing the solution, surrounded by a large volume of warm water, to cool slowly, an abundant deposit of octahedral crystals was obtained, which were, however, not quite so perfectly developed as those just described.

No analysis of these distinct crystals has as yet been made. Their further investigation will be undertaken directly.

## VIII.—Methods of Analysis.

*Drying.*—Preparations preceding **12** were dried in hydrogen at 110° C. to a constant weight. Those following were dried in air at 110° C. Carbon and hydrogen were determined by combustion in a platinum boat in open tube filled with copper oxide and containing in addition a layer of lead chromate and a roll of metallic copper. In the analyses preceding that of **12** the portions taken for each carbon determination were dried separately to a constant weight at 110° C. in a stream of hydrogen, and weighed in a closed tube. For those following, the entire sample was dried to a constant weight in air. By the latter method of drying, slightly higher percentages of hydrogen were obtained when the

substance analysed was in the form of a light hygroscopic powder; the dense preparations obtained from alkaline solutions, not being so hygroscopic, gave slightly lower percentages of hydrogen: the difference is attributable to moisture unavoidably absorbed by the dry, light powder during weighing and transferring to the combustion-tube, the analyses being made in very damp summer weather.

The accuracy of the determinations was repeatedly controlled by analyses made on pure sugar.

*Nitrogen.*—Nitrogen determinations were made by both the Kjeldahl and Dumas methods, in the manner customary in the Station Laboratory.[1] Preceding **12** the determinations were made upon the substance dried over sulphuric acid, a correction being applied for the moisture contained in the sample as found by drying it at 110° C. Subsequent determinations were made on the fully-dried sample.

*Sulphur.*—Sulphur was determined by fusion with sodium hydroxide and potassium nitrate, dissolving the fused mass in water, neutralising the alkali with a considerable excess of hydrochloric acid, evaporating to dryness to decompose nitrates and to remove excess of acid, dissolving the dry residue in six or seven hundred centimeters of water, allowing the solution to stand for twenty-four hours, filtering, heating to boiling, and precipitating with 3 cc. of a 10-per cent. solution of barium chloride.

In the analyses of **8**, evaporation to dryness was omitted, the barium sulphate being fused with sodium carbonate and reprecipitated. In all cases the ash of the preparations was carefully examined for sulphates, but none was found in any instance.

Blank experiments with the reagents proved them to be entirely free from sulphur.

*Ash.*—Ash was determined by combustion in a platinum crucible, no difficulty being experienced in burning any of the preparations except **18**.

## IX.—Summary of Results.[2]

1. The proteid body removed from freshly-ground oats by direct extraction with weak alcohol—first observed by Norton and

[1] Annual report Conn. Station, 1879, p. 124; and This Journal 2, 27.

[2] This summary is a revision of a part of Bulletin 105, issued by the Connecticut Agricultural Experiment Station, December, 1890, which contained some minor errors that are here corrected.

by him designated *glutin*—when dehydrated by absolute alcohol and dried over sulphuric acid, is a light yellowish powder, insoluble in pure water as well as in absolute alcohol, soluble in mixtures of alcohol and water, soluble also in dilute acids and alkalies, and from these solutions thrown down by neutralisation. Separated from its solution in alcohol of 60 per cent. by evaporating off the alcohol, it forms a yellowish, slimy mass. Its composition (as found for preparation **8**[1]) is given in the table, p. 338, under **I.** This substance is remarkable for its considerable content of sulphur, which is exceeded by that of keratin alone among the proteids, and is otherwise equaled only by that recorded in some analyses of serum-albumin. See p. 340.

2. When the substance described above is heated with dilute alcohol for some time, it coagulates and become insoluble in that liquid, but without apparent change of composition. **II** (p. 338) is the average of three accordant analyses of this coagulated form of the proteid, which is soluble in alcohol, made on preparations **7**, **4 A** and **4 B** (pp. 334–336).

Kreusler obtained this material from the oat, but what Ritthausen and he named *oat-gliadin* was a product of its further alteration by the chemical treatment to which it was subjected with a view to purification. (See pp. 340–341.)

3. When oats are first treated with water or 10-per cent. solution of common salt, before extraction with dilute alcohol, the proteid, soluble in alcohol, undergoes alteration, and a body of different composition and properties results. In the table, **III**, is the mean of closely-agreeing analyses of this substance made on preparations **9**, **10**, **11** and **12**; it is much more soluble in dilute alcohol than **I**, and is not coagulated or transformed into an insoluble modification. When wet with absolute alcohol, the moisture attracted from the air shortly renders it gummy and tenaciously adhesive, unlike **I**.

Its composition, as regards carbon, hydrogen, and nitrogen, is very near to that found by Dumas and Cahours, and also by v. Bibra, for *gliadin* or *plant-gelatin* (extracted by hot alcohol from wheat-gluten and remaining dissolved in the alcohol when cold).

4. The chief proteid extracted by cold 10-per cent. salt-solution behaves towards reagents like the *myosin-glubolin* from animal muscle, as first stated by Weyl. Contrary to Weyl's observations,

[1] *Vide* p. 338.

however, the coagulation-temperature (80°–100°) is much higher than that of animal myosin (55°–60°). This proteid appears to be the result of a transformation similar to that by which myosin is formed from myosinogen. Its composition—the average of several analyses on preparations **13** and **14**—is given under **IV**, and is very near to that of muscle myosin. The greatest proportion of this proteid extracted by salt-solution from the oat was 1.3 per cent. (See pp. 389–394.)

5. The proteid extracted, after complete exhaustion of the oats with alcohol of 0.9 sp. gr., by 10-per cent. salt-solution (analysis of Preparation **15** under **V**), and that dissolved out by dilute potash (analysis of Preparation **16** under **V** *a*), have so nearly the same composition as the globulin extracted by salt-solution directly, that they may be regarded as originally identical, **IV** representing the soluble form, **V** and **V** *a* the insoluble or "albuminate" modification. (See pp. 394–399.)

6. When ground oats are directly extracted by weak potash solution, without previous treatment with water or dilute alcohol, nearly the whole of the proteids is dissolved. The substance so extracted, after completely removing the body soluble in weak alcohol, is perhaps the same as that first designated *avenine* by Johnston and Norton, who extracted oats with dilute ammonia-water. Its composition, as indicated by analysis of a single preparation, **17**, is stated under **VI**. (See also pp. 399–400 and 403.)

7. When ground oats are exposed to the action of water, a large share of the proteids becomes insoluble in dilute potash solution, the amount so rendered insoluble increasing with the duration of the contact with water. One hour's treatment with water rendered one-half, twenty-four hours' treatment made two-thirds insoluble in $\frac{2}{10}$-per cent. solution of potash. The composition of the part soluble in potash, after action of water (and removal of the alcohol-soluble proteid), as found in analyses of Preparations **18** and **19**, the average of which is stated under **VII**, is the same as that of the globulin soluble in salt solution, **IV**. This proteid, obtained by extraction with potash, *after* the action of water, is probably the substance which Kreusler converted into his *Oat-Legumin* by the "purifying" process to which he subjected it. It is also the "protein body" which Norton extracted by weak ammonia and analysed. (See pp. 400–405.)

8. When ground oats are extracted with 10-per cent. sodium chloride solution heated at 65° C., a proteid separates on cooling, in the form of spheroids. This substance differs in composition and properties from that obtained by cold salt-extraction, as well as from all proteids hitherto described. It is soluble in pure water, precipitated from such solutions by a little sodium chloride, is again dissolved by a certain additional quantity, and is precipitated completely by saturation with this salt. In the presence of a little sodium chloride and acetic acid it is soluble in alcohol of 0.9 sp. gr. From solutions in distilled water, as well as from those in sodium-chloride brine, it has been obtained crystallised in regular octahedra. Analysis (of spheroids) under **VIII.** (See pp. 405–409.)

9. The aqueous extract of ground oats was found, in agreement with Norton and Kreusler, to contain very little proteid substance. The proteids thus dissolved appear to be, first, an *acid-albumin*; second, a *globulin* or *globulins* similar in reactions to that extracted by 10-per cent salt-solution, and third, a *proteose*. No true albumin was found in the water-extract. (See pp. 385–389.)

10. In the salt-extract a very small amount of a body was found, having the reactions of *albumin*, but not analysed (pp. 389–390).

## X.—Table of Composition of Proteids from the Oat-Kernel.

| | I. | II.[1] | III.[1] | IV. | V. | V *a*. | VI. | VII.[1] | VIII. |
|---|---|---|---|---|---|---|---|---|---|
| Carbon, | 53.06 | 53.10 | 53.70 | 52.34 | 52.48 | 52.45 | 53.49 | 52.49 | 52.22 |
| Hydrogen, | 6.94 | 6.91 | 7.00 | 7.21 | 6.94 | 6.92 | 7.01 | 7.10 | 6.98 |
| Nitrogen, | 16.38 | 16.49 | 15.71 | 16.88 | 16.85 | 16.63 | 16.39 | 17.11 | 17.85 |
| Sulphur, | 2.26 | } 23.50 | 1.76 | 0.88 | 0.57 | 0.81 | 0.99 | 0.80 | 0.77 |
| Oxygen, | 21.38 | } | 21.83 | 22.69 | 23.16 | 23.19 | 22.12 | 22.50 | 22.18 |
| | | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

The numbers over the analyses in the above table correspond with those of the paragraphs in the foregoing summary.

Laboratory of the Conn. Agricultural Experiment Station.

[1] Average of several analyses.

# THE DECOMPOSITION OF CHROMITE BY THE ELECTRIC CURRENT.

By Edgar F. Smith.

Two years ago I mentioned[1] that if finely divided chromite were suspended in melted alkali, and an electric current allowed to act upon the mixture, the mineral would undergo decomposition, its chromic oxide being changed to a soluble alkaline chromate. Details of the experiments made at that time were not published, because difficulties were encountered which necessitated a more careful study of the subject.

The early trials were made with a current of one ampère. The oxidation of the mineral was apparently finished in fifteen minutes, but the desire to reduce the time-factor, by increasing the strength of the acting current, led me astray. Many successful and unsuccessful decompositions were made before conditions were found which gave satisfactory results. Among other things it was observed that by using a strong current the latter seemed to act chiefly upon the iron oxide and the caustic alkali, liberating larger or smaller quantities of the metal from each. After the decomposition of the mineral had proceeded to a certain extent, the action just mentioned seemed to commence, and undecomposed chromite always remained. Without describing the various modifications that were tried in the hope of attaining success, I will briefly outline the method which finally gave, and has continued to give, good results.

*Method of Oxidation.*—Melt 30–40 grams of stick potash in a nickel crucible, $1\frac{7}{8}$ inches high and 2 inches wide; heat gently until the excess of moisture has been expelled. The crucible is then placed upon a heavy copper-wire ring, attached to the anode of a storage-cell.[2] A small flame is held under the crucible during the entire decomposition. The weighed mineral is carefully brushed from a watch-crystal upon the liquid alkali, the crucible covered with a perforated glass, and the platinum rod, attached to the cathode of the battery, lowered into the melted mass. Set the current in operation; particles of the melted material are thrown

[1] J. Franklin Inst., April, 1889; Ber. d. chem. Ges. **22**, 1019.

[2] A picture of the apparatus arranged for a decomposition of this kind is given in Ber. d. chem. Ges. **23**, 2276; J. Franklin Inst. **130**, 145; and in the writer's "Electrochemical Analysis," p. 111.

against the under-side of the cover-glass, but the liquid soon collects in drops and falls back into the crucible, thus returning any traces of ore that at first may have been projected from the crucible by the bursting of the gas-bubbles.

Experience has demonstrated that it is best to make the crucible the anode; but before finally interrupting the current, the latter should be reversed by means of a commutator, which, together with an ampèremeter, is always in circuit. The current is reversed for this reason: metallic iron is deposited upon the cathode and may enclose particles of the ore, removing them from the oxidising field. By reversing the current the imprisoned material is set free and exposed to oxidation.

The quantity of mineral taken for a determination may vary from 0.1–0.5 gram. It should be quite fine, although it was never as finely divided as seemed necessary for complete decomposition by other methods. The current employed in the oxidation of the material *should not exceed one ampère* for the quantities given above. As to the time required for the decomposition, it can only be said that while the greater part of the mineral is unquestionably decomposed in fifteen minutes, it is best to let the current act for a period of thirty to forty minutes. Much depends upon how refractory the sample under analysis may prove to be. Chromites vary in this respect. It is a difficulty with which all methods must contend. Hence, at least one half-hour should be allowed for an oxidation by the electrolytic method. As the decomposition advances the melted alkali acquires a yellow color. The portion that creeps to the edge of the crucible and about the cover-glass has the appearance of cauliflower.

The current can now be interrupted. Raise the platinum rod (the cathode) from the liquid, remove the cover-glass carefully from the crucible with a nickel tongs, rinse its under-surface with water, collecting the latter in a beaker. The crucible is lifted from the ring supporting it and allowed to cool. The platinum cathode should be rinsed with water. It will show a black appearance, due to the metallic iron that had been deposited upon it. It is my plan to dissolve off the iron with dilute hydrochloric acid, to assure myself that undecomposed ore was not present.

When the nickel crucible is cold, place it in a beaker of 300 cc. capacity, cover with water, and digest upon a warm iron plate.

In a few minutes the contents of the crucible can be entirely removed. The liquid now contains the excess of alkali, sodium aluminate, silicate, manganate, chromate, etc. After digesting for some time upon a warm iron plate, filter off the insoluble portion and thoroughly wash it with boiling water. The yellow alkaline solution is now ready for the determination of the chromium.

Treat the residue with warm hydrochloric acid. It should dissolve. It may happen that when quartz is present in the ore there will be a slight residue, but this is immaterial, provided it is not dark in color. In the analyses given later, the oxides remaining after treating the fused mass with water *dissolved completely* in warm hydrochloric acid.

*Chromium Determination.*—This may be made either gravimetrically or volumetrically. In the later determinations I used the volumetric method almost exclusively. To this end the chromate solution was acidulated with sulphuric acid, and a weighed portion of ferrous sulphate added, the excess being determined with a standardised bichromate solution. Potassium ferricyanide served as an indicator. In several instances the excess of ferrous salt was determined with potassium permanganate. This course, however, was abandoned, as I failed to read the end-reaction with sufficient accuracy. By using the volumetric method, I believe it possible for the analyst to decompose a chrome ore and determine the chromium content within the space of an hour. Several crucibles can be connected up in the same circuit, and a series of determinations made quite rapidly and with as much accuracy as by any one of our present methods.

*Results.*

1. 0.1080 gram chromite gave 51.77 per cent. $Cr_2O_3$.
2. 0.4787 gram chromite gave 51.55 per cent. $Cr_2O_3$.
3. 0.2729 gram chromite gave 51.23 per cent. $Cr_2O_3$.
4. 0.1085 gram chromite gave 51.74 per cent. $Cr_2O_3$.
5. 0.4184 gram chromite gave 52.02 per cent. $Cr_2O_3$.

By the Dittmar method of decomposition a sample of the same ore gave 51.70 per cent. $Cr_2O_3$.

A second chromite, decomposed by the current, gave as follows:

6. 0.1515 gram chromite gave 48.01 per cent. $Cr_2O_3$.
7. 0.2029 gram chromite gave 48.34 per cent. $Cr_2O_3$.

8. 0.3174 gram chromite gave 48.21 per cent. $Cr_2O_3$.

9. 0.2071 gram chromite gave 48.38 per cent. $Cr_2O_3$.

A portion of the ore oxidised by the Genth method and the chromic oxide determined gravimetrically gave 48.50 per cent. $Cr_2O_3$.

A light-colored chromite powder gave the following results:

10. 0.2012 gram chromite gave 32.00 per cent. $Cr_2O_3$.

11. 0.2130 gram chromite gave 31.50 per cent. $Cr_2O_3$.

A gravimetric determination of the chromium, after decomposition by the method of Dr. Genth, gave 31.90 per cent. $Cr_2O_3$.

A fourth specimen of chromite showed the following percentage of chromic oxide, after decomposition by the current:

12. 0.2295 gram chromite gave 42.20 per cent. $Cr_2O_3$.

13. 0.2225 gram chromite gave 41.70 per cent. $Cr_2O_3$.

By the Genth method this ore gave 41.30 per cent. $Cr_2O_3$, while 41.98 per cent. $Cr_2O_3$ was found after a decomposition made by the Dittmar method.

The writer would here acknowledge his indebtedness to Mr. D. C. Wallace for the care and skill with which he conducted the many oxidations that were considered desirable before publishing the facts contained in this communication.

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## ELECTROLYTIC SEPARATIONS.

BY EDGAR F. SMITH AND FRANK MUHR.

Having found after many trials that gold could be completely precipitated by the current from solutions of potassio-auric cyanide, steps were taken to ascertain from what metals it could be separated. Former studies[1] showed that a current of one-hundredth ampère did not precipitate palladium from a solution containing an excess of potassium cyanide until after it had acted for a period of thirty-six hours. This behavior pointed to a possible separation of gold from palladium. These two metals were,

[1] This Journal **12**, 252.

therefore, experimented upon with the current, under the conditions indicated in the following table:

| | Gold present. Gram. | KCN. Grams. | Total Dilution. | Current in cc. OH gas per minute. | Palladium present. Gram. | Gold found. Gram. | Difference in percentage from theory. |
|---|---|---|---|---|---|---|---|
| 1. | 0.1162 | 1.50 | 150 cc. | 0.80 cc. | 0.1200 | 0.1162 | .. |
| 2. | " | " | " | " | " | 0.1163 | +0.08 |
| 3. | " | " | " | " | " | 0.1162 | .. |
| 4. | " | " | " | " | " | 0.1163 | +0.08 |
| 5. | " | " | " | 0.40 cc. | " | 0.1160 | —0.17 |
| 6. | " | " | " | " | " | 0.1156 | —0.52 |
| 7. | " | " | " | 1.00 cc. | " | 0.1164 | +0.17 |
| 8. | " | " | " | " | " | 0.1158 | —0.34 |

The time allowed for the precipitation of the gold varied from twelve to fourteen hours. Cold and hot water were used for washing the precipitated metal. The deposits were made directly upon the platinum dishes. The gold was subsequently dissolved off by placing dilute potassium cyanide in the vessels and then connecting the latter with the anode of a feeble Crowfoot battery.[1] The gold cyanide solution was decomposed with *aqua regia* and, after obtaining gold chloride, this was carefully examined for palladium. None was discovered.

*Gold from Copper.*—The previous electrolytic separation of silver from copper[2] indicated that gold might perhaps be separated with equal facility from the latter metal, inasmuch as the complete deposition of the gold did not require a current much stronger than that necessary for the precipitation of silver. The separation proved successful under the conditions given below:

| | Gold present. Gram. | KCN. Grams. | Total Dilution. | Current in cc. OH gas per minute. | Copper present. Gram. | Gold found. Gram. | Difference in percentage from theory. |
|---|---|---|---|---|---|---|---|
| 9. | 0.1725 | 1.5 | 150 cc. | 0.8 cc. | 0.1250 | 0.1725 | .. |
| 10. | 0.1725 | 3.5 | " | 0.4 cc. | " | 0.1728 | +0.17 |
| 11. | " | " | " | " | " | 0.1720 | —0.29 |
| 12. | " | " | " | " | " | 0.1735 | +0.50 |
| 13. | " | 3 | " | 0.8 cc. | " | 0.1736 | +0.60 |

Copper was not detected in the precipitated gold. The time required for the precipitation, washing and drying of the deposits was the same as in the separation of gold from palladium.

*Gold from Cobalt.*—In the electrolytic separation of mercury

[1] J. Franklin Inst. **131**, 300; J. Anal. Chem. **5**, 205.
[2] J. Franklin Inst. **129**, 241.

from cobalt, and of silver from cobalt,[1] satisfactory results were obtained only after the quantity of alkaline cyanide present had been reduced to 3 grams. This quantity was used in the following separations, although its increase was not accompanied by the difficulties attending the separation of the metals to which reference has just been made. Indeed, in this particular case no special effort was required in order to get satisfactory results.

| | Gold present. Gram. | KCN. Grams. | Total Dilution. | Current in cc. OH gas per minute. | Cobalt present. Gram. | Gold found. Gram. | Difference in percentage from theory. |
|---|---|---|---|---|---|---|---|
| 14. | 0.1792 | 3 | 150 cc. | 0.6 cc. | 0.15–0.20 | 0.1789 | —0.11 |
| 15. | " | " | " | " | " | 0.1799 | +0.49 |
| 16. | " | " | " | " | " | 0.1794 | +0.11 |
| 17. | " | " | " | " | " | 0.1799 | +0.49 |

The current acted upon the double cyanides through the night.

*Gold from Nickel.*—This separation can be made without difficulty, as is proved by the four trials given below.

| | Gold present. Gram. | KCN. Grams. | Total Dilution. | Current in cc. OH gas per minute. | Nickel present. Gram. | Gold found. Gram. | Difference in percentage from theory. |
|---|---|---|---|---|---|---|---|
| 18. | 0.1725 | 2½ | 150 cc. | 0.5 cc. | 0.1373 | 0.1724 | —0.05 |
| 19. | 0.1725 | 3 | " | 1.0 cc. | " | 0.1718 | —0.49 |
| 20. | 0.1467 | 2½ | " | 0.5 cc. | " | 0.1462 | —0.34 |
| 21. | " | " | " | " | " | 0.1460 | —0.48 |

The electrolytic separation of mercury from zinc in cyanide solution, as well as cadmium and silver from the latter metal, occurred without any special difficulty.

*Gold from Zinc.*—The current to decompose the double cyanide of zinc and potassium must yield at least 2–3 cc. electrolytic gas per minute, so that special precautions in regard to the following separation need not be given:

| | Gold present. Gram. | KCN. Grams. | Total Dilution. | Current in cc. OH gas per minute. | Zinc present. Gram. | Gold found. Gram. | Difference in percentage from theory. |
|---|---|---|---|---|---|---|---|
| 22. | 0.1792 | 3 | 150 cc. | 0.5 cc. | 0.2000 | 0.1796 | +0.22 |
| 23. | " | " | " | 0.6 cc. | " | 0.1797 | +0.27 |
| 24. | " | " | " | " | " | 0.1790 | —0.11 |
| 25. | " | " | " | " | " | 0.1798 | —0.33 |

It will be noticed in the preceding experiments that the gold is either slightly in excess or below the theoretical amount. This cannot be due to incomplete precipitation or to the co-precipitation of the other metal present in the double cyanide solution, for

[1] J. Franklin Inst. **129**, 239, 244.

in each of the preceding cases careful search was made for all the metals operated upon; the variations from the theoretical requirements must therefore be attributed to some other cause. The precipitations were made in platinum dishes; these varied in weight from 60–71 grams. Care was not taken to weigh at any particular period of the day, nor were the precipitating-vessels allowed any definite time for drying, etc.

The battery used to precipitate the gold consisted of 6–10 Crowfoot cells. The poles, during precipitation, were 1½ inches apart. Cover-glasses were placed over the electrolysed solution to prevent loss and to exclude dust. The latter can easily cause the metal precipitated to collect in little spongy masses, which are readily detached upon washing. To determine when the gold was fully precipitated, the level of the liquid in the dish was raised by the addition of distilled water. Before the current was finally interrupted the poles were brought more closely together, and the electrolytic action continued for a half-hour longer. It is better to do this than to remove a portion of the liquid and apply some special test to it.

It is scarcely necessary to add that if the clean platinum surface remains uncoated, the precipitation of metal is finished.

The current of the strength given in the preceding experiments *does not precipitate* platinum either from hot or from cold solution of its double cyanide when an excess of alkali cyanide is present. In this respect platinum resembles palladium. This behavior prompted us to attempt, with good results, the separations recorded in the following lines:

*Gold from Platinum.*

| | Gold present. Gram. | KCN. Grams. | Total Dilution. | Current in cc. OH gas per minute. | Platinum present. Gram. | Gold found. Gram. | Difference in percentage from theory. |
|---|---|---|---|---|---|---|---|
| 26. | 0.1467 | 2½ | 150 cc. | 1 cc. | 0.2433 | 0.1474 | + 0.48 |
| 27. | " | " | " | " | " | 0.1474 | " |
| 28. | " | " | " | " | " | 0.1468 | + 0.05 |
| 29. | " | " | " | " | " | 0.1461 | — 0.40 |

*Silver from Platinum.*—In the first trials of the separation of gold from platinum the dishes were coated with a thick layer of metallic silver. When the platinum double cyanide solution was electrolysed, platinum metal was deposited upon the silver. Its precipitation was, however, incomplete; furthermore, silver

passed into solution. This behavior seemed to us to promise little in the way of separating silver from platinum. However, after dissolving definite amounts of salts of the two metals and adding an excess of potassium cyanide, the solution was directly electrolysed, when the silver separated upon the dish, perfectly free from platinum. Currents of greater strength than that given in the recorded experiments will throw out traces of platinum. It shows itself in dark spots, distributed over the white silver surface. In such cases a black, though minute, residue will always be noticed when the precipitated silver is dissolved in dilute nitric acid. The results were as follows:

| | Silver present. Gram. | KCN. Grams. | Total Dilution. | Current in cc. OH gas per minute. | Platinum present. Gram. | Silver found. Gram. | Difference in percentage from theory. |
|---|---|---|---|---|---|---|---|
| 30. | 0.0770 | 2½ | 150 cc. | 1 cc. | 0.2433 | 0.0772 | + 0.25 |
| 31. | " | " | " | " | " | 0.0768 | — 0.25 |

*Cadmium from Platinum.*—Reference to a paper by Smith and Frankel [1] will disclose the fact that they failed to obtain an electrolytic separation of silver and cadmium from palladium. The separation of silver from platinum succeeds if conducted under the conditions outlined in the preceding example, but the separation of cadmium from platinum resulted negatively, although the current was reduced to 0.2 cc. oxy-hydrogen gas per minute. The precipitated cadmium always contained platinum. We also found that if the platinum dishes were coated with cadmium and the double cyanide of platinum and potassium electrolysed in these vessels, the platinum metal was deposited quite rapidly; cadmium, however, invariably dissolved. This behavior was similar to that observed in the attempted electrolytic separation of cadmium from palladium and from nickel.

Platinum, palladium and nickel show many points of similarity in deportment when their double cyanide solutions are electrolysed.

*Mercury from Platinum.*—This separation is as readily made as that of mercury from palladium and nickel.

| | Mercury present. Gram. | KCN. Grams. | Total Dilution. | Current in cc. OH gas per minute. | Platinum present. Gram. | Mercury found. Gram. | Difference in percentage from theory. |
|---|---|---|---|---|---|---|---|
| 32. | 0.1902 | 2.5 | 150 cc. | 0.2 cc. | 0.2433 | 0.1900 | — 0.10 |
| 33. | " | " | " | " | " | 0.1904 | + 0.10 |
| 34. | " | " | " | " | " | 0.1905 | + 0.15 |
| 35. | " | " | " | " | " | 0.1910 | + 0.41 |

[1] This Journal **12**, 428.

The separations of gold, silver and mercury from platinum were carried out in the cold. The depositions were made in the same platinum vessels used in the experiments recorded in the first part of this communication. The deposited metals were treated as mentioned in previous papers. The time of precipitation occupied from fourteen to sixteen hours.

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## THE CONSTITUTION OF BENZOQUINONE.—II.

BY J. U. NEF.

In a recent paper on this subject,[1] I presented a series of experiments which were so definitely and exclusively in favor of Fittig's formula for quinone, that it seemed as if a doubt concerning the constitution of this substance was no longer admissible. It has been asserted,[2] however, that the proof that quinone dibromide has its bromine atoms in the ortho-position is not positive.

The existence of a para-binding in hydrogenated benzene derivatives has already been rendered very improbable by v. Baeyer's work on benzene,[3] and my own experiments in the anilic acid series[4] have rendered the view based upon the presence of a para-binding in quinone compounds highly improbable. For this reason I pointed out that rhodizonic acid could not possess the constitution first given it by Nietzki, and this conclusion has been confirmed by a recent paper of Nietzki[5] on this subject. There is, therefore, at present not a single fact which points in any way to the existence of a para-binding in benzene or in its hydrogenated derivatives.

That, therefore, quinone dibromide has its bromine atoms in the ortho- and not in the para-position is highly probable, especially since the diagonal ketone-formula was proved untenable by reason of experiments with the anilic acids.

[1] This Journal **12**, 463.

[2] Claus: J. prakt. Chem. [2] **42**, 463; Nietzki: Ber. d. chem. Ges. **23**, 3141; Kehrmann: J. prakt. Chem. [2] **43**, 106.

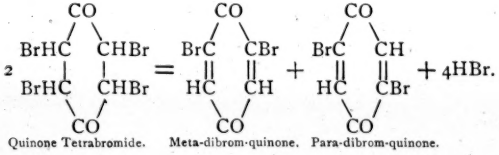
[3] Ann. Chem. (Liebig) **251**, 257, etc.

[4] This Journal **12**, 467.

[5] Ber. d. chem. Ges. **23**, 3136.

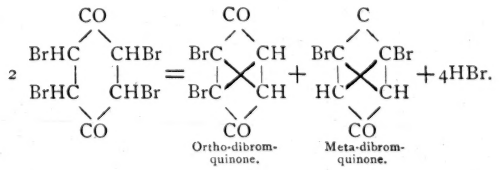
Furthermore, the behavior of quinone di- and tetrabromide towards zinc dust and acetic acid is so analogous to the behavior of v. Baeyer's addition-products of hydrogenated terephthalic acids,[1] especially of $\Delta 2,5$ dihydro-terephthalic acid di- and tetrabromide,[2] that there can be little doubt that they are of similar constitution.

The ortho-position of the bromine atoms in quinone dibromide follows also from the behavior of quinone tetrabromide. The latter substance is converted, as I have shown, by boiling with alcohol and water, into meta- and para-dibrom-quinone:




Quinone Tetrabromide. Meta-dibrom-quinone. Para-dibrom-quinone.

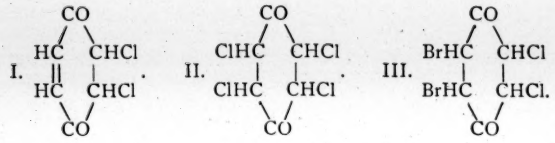
To form these two substances the hydrobromic acid must be split off from ortho-positions: if this splitting-off were to take place from the para-position, only ortho- and meta-dibrom-quinone could be formed:




Ortho-dibrom-quinone. Meta-dibrom-quinone.

It follows, therefore, that the hydrobromic acid in quinone tetrabromide is split off from ortho-positions, since not a trace of ortho-dibrom-quinone is formed, but, on the contrary, much para-dibrom-quinone. The conclusion that the addition of bromine takes place in ortho-positions is therefore highly probable. That this is the case I am now able to give further and absolutely conclusive proofs. Mr. T. H. Clark has, under my direction, studied the action of halogens on quinone, and has obtained products whose constitution is to be represented as follows:

[1] Ann. Chem. (Liebig) **245**, 103; **251**, 257. [2] Herb: Ibid. **258**, 11.



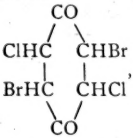
I. Quinone dichloride—pale yellow; melting-point, 143°; distils without change.

II. Quinone tetrachloride—colorless; sublimes without melting and without decomposition.

III. Quinone dichloride-dibromide—colorless; melting-point, 195°.

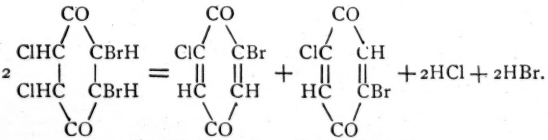
These substances are not affected by strong nitric acid, and show the same behavior in all details as the two bromine addition-products already described. The only difference is the greater stability of the chlorine derivatives, which sublime without decomposition.

That the quinone dichloride-dibromide obtained by the action of bromine on quinone dichloride has the constitution represented by the formula above given, and not that represented by the



formula , is proved as follows:

The substance is decomposed by boiling with alcohol and water into about equal parts of meta- and para-chlor-brom-quinone:

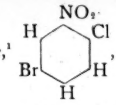


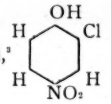
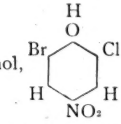
Meta-chlor-brom-quinone. Para-chlor-brom-quinone.

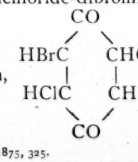
The latter consists of plates which melt at 172°; the former, of needles melting at 113°.

These two isomers were easily separated by fractional crystallisation from ether, wherein the para-compound is the less soluble.

Besides these two isomers no other product was found. That the chlor-brom-quinones obtained have the above-represented constitution was proven by the direct synthesis of these two substances, as follows:

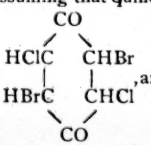
(*a*). Para-chlor-brom-nitrobenzene,[1]  , was reduced by means of zinc dust and acetic acid to chlor-brom-anilin (melting-point, 43°), and this substance gave, on boiling with manganese dioxide and dilute sulphuric acid, para-chlor-brom-quinone, identical in every particular with the above product, and with a chlor-brom-quinone obtained by Levy and Schultz[2] from chlor-quinone.

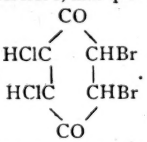
(*b*). Chlor-para-nitro-phenol,[3]  , was converted into brom-chlor-para-nitro-phenol,  , (melting-point, 138°), and this reduced with tin and hydrochloric acid. The tin double-salt of chlor-brom-amido-phenol separates out in flat needles, which, when converted into chlor-brom-amido-phenol sulphate, and boiled with dilute sulphuric acid and manganese dioxide, gave almost quantitatively meta-chlor-brom-quinone, crystallising in needles whose melting-point is 113°, identical in every respect with the corresponding quinone obtained from quinone dichloride-dibromide.

(*c*). If quinone dichloride-dibromide have its two chlorine atoms in the para-position,  , and it be decomposed by

[1] Körner: Jsb. Chem. 1875, 325. [2] Ann. Chem. (Liebig) **210**, 260.
[3] Kollrepp: Ibid. **234**, 2.

alcohol and water, assuming the splitting off of halogen hydride to take place from ortho-positions, in great part, if not exclusively, the resulting compound would be para-dichlor-quinone (melting-point, 164°), since the chlorine atoms are more firmly bound than the bromine atoms. The other products that might be formed would be para-dibrom-quinone (melting-point, 188°), and meta-chlor-brom-quinone. In the above reaction, however, not a trace of para-dichlor- or of para-dibrom-quinone was found.

(*d*). Assuming that quinone dichloride-dibromide have the constitution  , and that halogen hydride be split off from para-positions, only ortho-chlor-brom-quinone and meta-chlor-brom-quinone could be formed.

From the above facts it follows conclusively, therefore, that quinone dichloride-dibromide has the constitution  .

The addition of halogens to quinone takes place, moreover, in ortho-positions, and the splitting off of halogen hydride from the addition-products takes place invariably from ortho-positions. These facts can be explained only by Fittig's formula for quinone: their explanation is impossible on the assumption of the diagonal ketone-formula of Claus, and therefore this formula no longer deserves consideration.

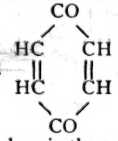
The publication of the details of the experimental work of Mr. Clark will take place in this Journal as soon as sufficient material has accrued for a thesis.

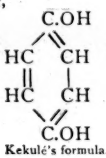
I desire to reserve for this laboratory, for the present, the study of the addition-products of quinone.[1] It is already highly proba-

[1] I very much regret the fact that Kehrmann's comments (J. prakt. Chem. [2] **43**, 106) are full of inaccuracies, as any one can see who will take the trouble to go over the literature of the subject. In my paper (Ber. d. chem. Ges. **20**, 2030) I stated definitely that "Die Versuche über die Ersetzbarkeit der Chloratome im Chloranil *durch verschiedene andere Radikale* werden fortgesetzt." Kehrmann's statement, therefore, shows that he has not properly examined into the subject. A number of Kehrmann's recent publications have been merely an application of my nitrite-reaction to other chlorinated quinones. (See Ber. d. chem. Ges. **21**,

ble that the peculiar action of hydrochloric and hydrobromic acid on quinones is due to the intermediary formation of addition-products which have been overlooked.

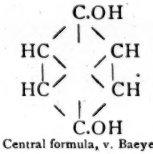
Finally, it is necessary to discuss briefly a subject brought forward by Nietzki,[1] namely, the conversion of quinone into hydroquinone, and its bearing on the constitution of benzene.

Since quinone possesses the formula  , a rearrangement of the double bonds must take place in the conversion into hydroquinone,



Kekulé's formula.

or



Central formula, v. Baeyer.

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1777; J. prakt. Chem. [2] **39**, 377; **40**, 179). When, however, Kehrmann began experiments with chloranil itself, it seemed to me he was directly interfering with my work.

In my paper on Quinone (This Journal **12**, 470) I distinctly cite that Kehrmann had already obtained ethyl dihydro-chloranilate. He, however, had obtained two isomers which he stated could be converted into one another. This statement, he has since shown, was incorrect (J. prakt. Chem. [2] **43**, 260).

I overlooked Kehrmann's observation that ethyl chloranilate is saponified by dilute alkalies. The isolation of a diacetyl derivative of chloranilic acid, which had long been sought for in vain, it seems to me is the most conclusive proof of the presence of two hydroxyl groups in the anilic acids. That the existence of tautomerism in this group has been assumed is evident to any one who has read Hantzsch's work on this subject. Furthermore, I had myself—because of the fact that it is impossible to oxidise away the two hydrogen atoms of the anilic acids, and because ethyl chloranilate does not take up bromine (This Journal **11**, 20)—put forward the hexamethylene formula, $\mathrm{X}\!>\!\mathrm{C}\!<\!\overset{\mathrm{CO-CO}}{\mathrm{CO-CO}}\!>\!\mathrm{C}\!<\!\mathrm{X}$, for the anilic acids. The behavior of chloranilic acid salts towards bromine (This Journal **12**, 475), whereby $\mathrm{Cl}\!>\!\mathrm{C}\!<\!\overset{\mathrm{CO-CO}}{\mathrm{CO-CO}}\!>\!\mathrm{C}\!<\!\mathrm{Cl}$ is unquestionably formed, points towards the hexamethylene formula for chloranilic acid salts, $\mathrm{Cl}\!>\!\mathrm{C}\!<\!\overset{\mathrm{CO-CO}}{\mathrm{CO-CO}}\!>\!\mathrm{C}\!<\!\mathrm{Cl}$.

To draw the conclusion from this reaction that chlor-anilic acid is $\mathrm{Cl}\!>\!\mathrm{C}\!<\!\overset{\mathrm{CO-CO}}{\mathrm{CO-CO}}\!>\!\mathrm{C}\!<\!\mathrm{Cl}$, is as justifiable as Victor Meyer's conclusion that sodic nitro-methane is $\mathrm{H_2C}\!<\!\mathrm{NO_2}$ because it forms with bromine $\mathrm{H_2C}\!<\!\mathrm{NO_2}$.

It is therefore apparent that the presence of two hydroxyl groups in chloranilic acid had to be proved beyond a doubt. That Kehrmann did not draw this conclusion from his results is evident, since he makes no mention of this question in his paper (J. prakt. Chem. [2] **40**, 370); and my work on the anilic acids (This Journal **11**, 20), as well as Hantzsch's (Ber. d. chem. Ges. **20**, 1308, **21**, 2439); and Levy and Jedlicka's work (Ann. Chem. (Liebig) **249**, 66), should have been known to him.

[1] Ber. d. chem. Ges. **23**, 3141.

That such a rearrangement of the valences should take place seems to me to be probable for the following reason:

It is impossible to oxidise to the corresponding quinone a number of carboxylated derivatives of hydroquinone, namely, hydroquinone-carboxylic acid or its ether;[1] tolyl-hydroquinone-carboxylic acid or its ether;[2] para-dioxy-terephthalic acid or its ethyl-ester.[3] It seems to me that these facts, which are in themselves very noteworthy, can only be explained when the conversion of hydroquinone into quinone is accompanied not only by a removal of the two hydrogen atoms, but also by a rearrangement of the valences of the carbon atoms forming the closed chain.

CHEMICAL LABORATORY, CLARK UNIVERSITY, *April*, 1891.

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**Contributions from the Chemical Laboratory of the Department of Agriculture, from H. W. Wiley.**

## XIX.—THE BIOLOGICAL FUNCTION OF THE LECITHINES.

### (*Preliminary Notice.*)

BY W. MAXWELL.

In an earlier stage of the study of the lecithines I showed, in an article entitled "On the Behavior of the Fatty Bodies, and the Role of the Lecithines during Normal Germination,"[4] that the inorganic phosphorus present in the mature seed, under the action of the processes occurring during incipient growth, becomes reorganised, and appears in the young plantlet in the organic form as a constituent of lecithine.

In the course of continuation of the researches, which have been extended or carried over from the vegetable to the animal organism, I have established the observation that the phosphorus which is present in the organic form as a lecithine in the egg of a hen, under the action of the process of incubation, becomes reorganised, and is found in the form of a mineral phosphate in the bone of the chicken.

[1] Ann. Chem. (Liebig) **237**, 35.
[2] Brünner: Monatsh. Chem. **2**, 464.
[3] Hantzsch and Löwy: Ber. d. chem. Ges. **19**, 26.
[4] This Journal **13**, No. 1.

In the light of the data to be given in a later contribution, of which this is a preliminary notice, it will be possible to indicate the course of the circulation of the element phosphorus in the three divisions of matter, and to show that it is (at least in great part) in the organic form of the lecithine or lecithines that the inorganic phosphorus of the mineral kingdom passes through the vegetable kingdom into the inorganic form, constituting the bone, of the animal kingdom.

## REVIEWS AND REPORTS.

GRUNDZÜGE DER THEORETISCHEN CHEMIE. Von Lothar Meyer. Breitkopf und Härtel, Leipzig, 1890. xii + 206 pp. 8vo.

The author of that great work, "Die Modernen Theorien der Chemie," has now given us an introduction to theoretical chemistry. In preparing it he has had in mind not only the needs of students, but of those who are interested in the advance of chemistry in a general way and yet have not the time to follow it in detail. His main effort has been to treat the subject in such a way as to give a general, broad, philosophic conception of it. As might have been expected, the author has admirably accomplished his object. The book is clear and thorough, and can be cordially recommended to those for whom it was written. It differs, of course, in some respects from other similar books that have recently appeared in Germany, while resembling them in other respects. It has most in common with Ostwald's "Grundriss der allgemeinen Chemie," as both emphasize the physical side of chemical phenomena. It is interesting to note that this conservative writer accepts in the main the ideas which have been put forward by Arrhenius, Van't Hoff, Ostwald and others, concerning electrolytic dissociation and osmotic pressure, and that in this introduction he devotes a number of pages to their treatment. It would be hard to find a clearer treatment of these subjects. In regard to the subject of stereo-chemistry, too, the author is in sympathy with the most advanced views. On page 98 he says: "Durch die Einführung der räumlichen, geometrischen Unterschiede in die Structurformeln der organischen Verbindungen haben nicht nur zahlreiche früher unerklärbar scheinende Isomerien eine vollkommen befriedigende Erklärung erfahren; sondern es sind auch noch zahlreiche Beziehungen zwischen der

Atomlagerung und dem Verhalten der Stoffe aufgefunden und aufgeklärt worden, so dass die erst im Jahre 1874 aufgestellte Hypothese vom unsymmetrisch gebundenen Kohlenstoffatome bereits zu den best begründeten Lehren der Chemie gezählt werden darf." The study of such books as this by teachers as well as by students will hasten the arrival of the day when chemistry will be studied as a branch of science. I. R.

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The Belgian Royal Academy of Science, Literature and Art sends invitations to the leading universities and scientific societies of all countries to join in fitly celebrating the fiftieth anniversary of M. Stas' membership of the Academy. It is proposed to present congratulatory addresses and a gold medal. Jean Servais Stas was born in 1813. He was a pupil of Dumas, and his first publication appeared in the Annales de Chimie et de Physique in 1838, on Phloridzin. He has published papers on subjects in organic and inorganic chemistry in the period from 1838 to 1874. He is best known by the one published in 1860 in the Bulletin of the Belgian Academy, containing the results of his long and patient studies of the atomic weights of the elements, which have furnished a standard for the subsequent work in this field. The English Royal Society gave him the Davy medal in recognition of the importance of this work.

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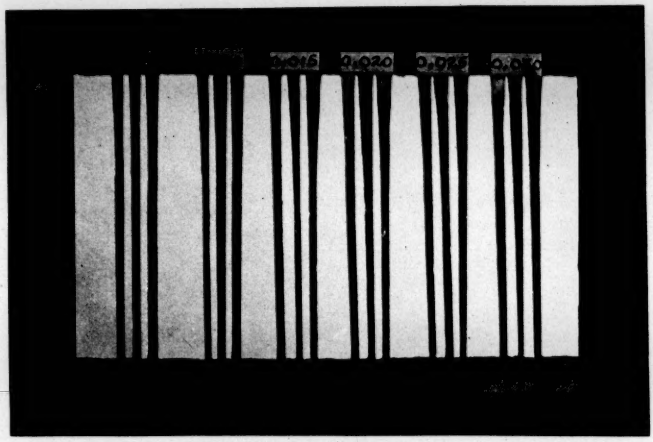
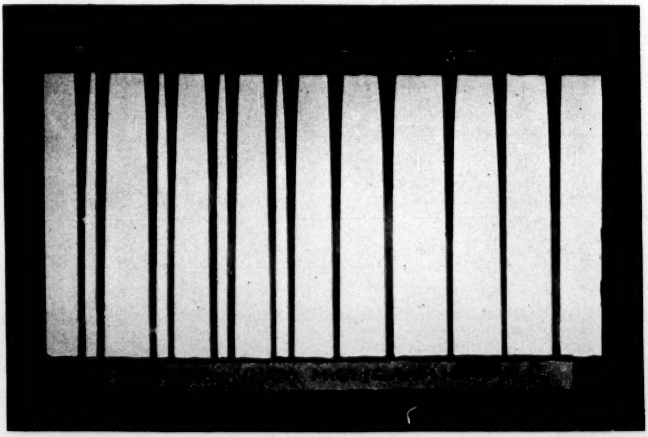
FRANCIS CLARK BLAKE died in Helena, Montana, February 21st. Mr. Blake was born in Andover, Mass., in 1854, he graduated at the Worcester Polytechnic Institute in 1876, and in 1877 was appointed Adjunct Professor of Chemistry at Lafayette College. In 1879 he resigned to enter the employ of the Pennsylvania Lead Company, where he remained as assistant superintendent and as superintendent till his death, devoting his energies to the improvement of this extensive refinery.

JAMES BUCKTON MACKINTOSH died in New York, April 15th. Mr. Mackintosh came to this country from England in 1873. His attention was chiefly given to analytical chemistry, especially to the study of the rare earths and of new minerals. He contributed several articles on analytical methods to this journal.

DR. CARL SCHAEDLER died in Berlin, April 24th. Dr. Schaedler was well known as an expert in technological chemistry, and as a writer of books, of which his "Technologie der Fette und Oele des Pflanzen- und Thierreichs" and "Technologie der Fette und Oele der Fossilien," are known as among the best books on vegetable, animal and mineral oils.





QUANTITATIVE DETERMINATION OF ARSENIC BY THE BERZELIUS-MARSH PROCESS STANDARD MIKRORS.